

# **Synthesis and Invitro Anti-Cancer Evaluation of Disubstituted 1,2,4 Thiazole Derivatives**

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

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## GENERAL REMARKS

1. Melting points (mp) were taken in open capillaries on **Thomas Hoover melting point apparatus** and are uncorrected.
2. The Infrared spectra were recorded in film or in potassium bromide disks on a **Bruker 398 spectrometer**.
3. The Proton Nuclear Magnetic Resonance spectra were recorded on a **DPX-500 MHz Bruker FT-NMR spectrometer**. The chemical shifts were reported as parts per million ( $\delta$  ppm) using tetramethylsilane (TMS) as an internal standard.
4. Mass spectra were obtained on a **JEOL-SX-102 instrument** using fast atom bombardment (FAB positive).
5. Elemental analyses were performed on a **Perkin-Elmer 2400 C, H, and N analyzer**.
6. Conventional reflux reactions were carried out by using **Scientific Microwave systems** (CATALYST SYSTEMS).
7. All reactions were monitored by thin layer chromatography on readymade silica gel plates (**Merck**).
8. Iodine was used as a developing agent. Spectral data (IR, NMR and MASS) confirmed the structures of the synthesized compounds and the purity of these compounds was ascertained by microanalysis.

## **CHAPTER -1**

### **1. INTRODUCTION**

Chemistry is a very broad subject, and can justly claim to encompass many aspects of the study of biological molecules. To most researchers in the cancer fields, the term 'chemistry' is often used in a much narrower way and is synonymous with the synthetic chemistry as a tool for the discovery of anticancer drugs.

#### **1.1. PHARMACEUTICAL CHEMISTRY**

Pharmaceutical Chemistry is an area of chemistry that deals with the structure, properties and reaction of compounds that contains carbon. Chemists in general and organic chemists in particular can create new molecules never before proposed which, if carefully designed, may have important properties for the betterment of the human experience. One of the main objectives of organic and medicinal chemistry is the design, synthesis and production of molecule having value as human therapeutic agents<sup>1</sup>. The department of pharmaceutical chemistry is to impart in depth knowledge about all the chemical aspects of drugs and natural products, such as the structure, synthesis, isolation and structural activity relationship with the pharmacological activity.

#### **Medicinal chemistry**

Medicinal chemistry and bioorganic chemistry is concerned with the design, synthesis and analysis of the relationship between molecular structure and

biological activity for compounds that can be used for the care or treatment of disease<sup>1</sup>. In medicinal chemistry, the chemist attempts to design and synthesis a medicine or pharmaceutical agent which will benefit humanity. Such a compound could be called as a ‘drug’.

Medicinal chemistry is a part of pharmaceutical chemistry. Medicinal chemistry is discipline at the intersection of chemistry and pharmacology involved with designing, synthesizing and developing pharmaceutical drugs. Medicinal chemistry involves the identification, synthesis and development of new chemical entities suitable for therapeutic use. It also includes the study of existing drugs, their biological properties and their quantitative structure activity relationship (QSAR).

Medicinal chemistry is the application of chemical research techniques to the synthesis of pharmaceuticals. During the early stages of medicinal chemistry development, scientist were primarily concern with the isolation of the medicinal agents founds in plants. Today, scientists in this field are also equally concerned with creation of new synthetic drug compounds. Medicinal chemistry almost always geared towards drug discovery and development

The first step is pharmaceutical focused on quality aspects of medicines and aims to assure fitness for the purpose of medicinal products. Medicinal chemistry is a highly interdisciplinary science combining organic chemistry with biochemistry, computational chemistry, pharmacology, pharmacognosy, molecular biology, statistics and physical chemistry.

The second step of drug discovery involves the synthetic modification of the hits in order to improve the biological properties of the compound pharmacophore.

**Heterocyclic chemistry** is the chemistry branch dealing exclusively with synthesis, properties and application of heterocycles. Heterocyclic compound is an organic compound that contains a ring structure containing atom in addition to carbon, such as sulfur, oxygen or nitrogen as part of the ring. They may be either simple aromatic ring or non-aromatic rings. Some heterocyclic compounds are known as carcinogens. Researches have show that heterocyclic amines are the Carcinogenic chemicals.

A heterocyclic compound is one which possesses a cyclic structure with at least two different kinds of hetero atoms in the ring. Nitrogen, oxygen, and sulphur are most common hetero atoms. Heterocyclic compounds are very widely distributed in nature and are essential to life in various ways.

### **1.2. 1, 3, 4- Thiadiazole<sup>2</sup>**

Many infectious diseases once considered incurable and lethal are now amenable. Today a major worldwide problem is resistance towards the available drug therefore in order to deal with resistance new compounds has to be synthesized. The resistance towards available drugs is rapidly becoming a major worldwide problem. The need to design new compounds to deal with this resistance has become one of the most important areas of research today. Thiadiazole is a versatile moiety that exhibits a wide variety of biological activities. Thiadiazole moiety acts as “hydrogen binding domain” and “two-electron donor system”. It also acts as a

constrained pharmacophore. Many drugs containing thiadiazole nucleus are available in the market such as acetazolamide, methazolamide, sulfamethazole, etc. Thiadiazole can act as the bio-isosteric replacement of the thiazole moiety. So it acts like third and fourth generation cephalosporins, hence can be used in antibiotic preparations. Thiadiazole is a 5-membered ring system containing two nitrogen and one sulphur atom. They occur in nature in four isomeric forms viz. 1,2,3-thiadiazole; 1,2,5-thiadiazole; 1,2,4-thiadiazole and 1,3,4-thiadiazole.

Thiadiazole derivatives possess interesting biological activity probably conferred to them by the strong aromaticity of this ring system, which leads to great in vivo stability and generally, a lack of toxicity for higher vertebrates, including humans. When diverse functional groups that interact with biological receptors are attached to this ring, compounds possessing outstanding properties are obtained.

### **1.3. Chemistry of Thiadiazole moiety:**

A series of thiadiazole have been synthesized using an appropriate synthetic route and characterized by elemental analysis and spectral data. There are various types of thiadiazole rings are present:

- 1, 2, 4-Thiadiazole
- 1, 3, 4-Thiadiazole
- 1, 2, 5-Thiadiazole
- 1, 2, 3-Thiadiazole

**1, 2, 4-Thiadiazole moiety:**

1,2,4 –Thiadiazole moiety contain sulfur at position -1, and two nitrogen atom at position -2 & position -4. The photochemistry of 1, 2, 4-thiadiazoles is of interest because the ring system can be viewed as a combination of a thiazole and an isothiazole <sup>1</sup>.

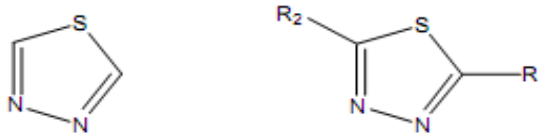


Therefore, 1, 2, 4-thiadiazoles would be expected to undergo phototransposition reaction, via sulfur migration around four sides of the photochemically generated bicyclic intermediates, and photocleavage of the S-N bond similar to those of thiazoles and isothiazoles.

**1, 3, 4 –Thiadiazole moiety:**

1,3,4- Thiadiazole moiety contain a heterocyclic nucleus in which sulfur present at position -1, and two nitrogen atom at position -3 & position -4.



**1, 2, 5-Thiadiazole moiety:**

1,2,5- Thiadiazole moiety contain a heterocyclic nucleus in which sulfur present at position -1, and two nitrogen atom at position -2 & position -5.

**1, 2, 3-Thiadiazoles moiety:**

1,2,3- Thiadiazole moiety contain a heterocyclic nucleus in which sulfur present at position -1, and two nitrogen atom at position -2 & position -3.



Moreover, much interest has also been focused on the cardiogenic, diuretic and herbicidal activities displayed by compounds incorporating this heterocyclic system<sup>1</sup>.

Thiadiazole is a heterocyclic compound containing both two nitrogen atom and one sulfur atom as part of the aromatic five-membered ring. It is a versatile moiety that exhibits a wide variety of biological activities. Thiadiazole moiety acts as —hydrogen binding domain and —two-electron donor system<sup>2</sup>. Thiadiazoles act as bioisosteric replacement of thiazole moiety. It is also bioisosteres of oxadiazole, oxazole and benzene. Substitution of these heterocycles with a thiadiazole typically

leads to analogues with improved activities because the sulfur atom imparts improved liposolubility<sup>3</sup>.

Thiadiazole occur in nature in four isomeric forms as 1,2,3-thiadiazole; 1,2,5-thiadiazole; 1,2,4-thiadiazole and 1,3,4-thiadiazole. The most fully investigated of these being the 1,2,4- and 1,3,4-thiadiazoles. Differently substituted thiadiazole moieties have different activity.

Isomers of thiadiazole compounds that contain thiadiazole ring are acetazolamide, methazolamide, sulfamethazole, etc. Other thiadiazole containing drugs include, cefazolin sodium (CFZL; and cefazedone (CFZD;)—first-generation cephalosporins; timolol—a nonselective  $\beta$ -adrenergic receptor blocker used for the treatment of hypertension, angina, tachycardia and glaucoma; xanomeline—a selective agonist of muscarinic acetylcholine receptor subtypes M1 and M4 and megazolan anti-parasitic drug<sup>4</sup>. Newly synthesized compounds are SCH-202676 in 2001 as a promising allosteric modulator of G-protein coupled receptors in 1998, KC 12291 as cardio protective action and in 2002 the small heterocyclic thiadiazolidinones (TDZD) as the first non-ATP competitive glycogen synthase kinase 3 $\beta$  inhibitors<sup>5</sup>.

#### **1.4. Properties of Thiadiazole**

It is a clear to yellowish liquid with a pyridine like odor. It is soluble in alcohol and ether and slightly soluble in water. Thiadiazoles carrying mercapto, hydroxyl and amino substituent's can exist in many tautomeric forms. Metal complexes having 1, 3, 4- thiadiazole nucleus has been used as anti- corrosion paints

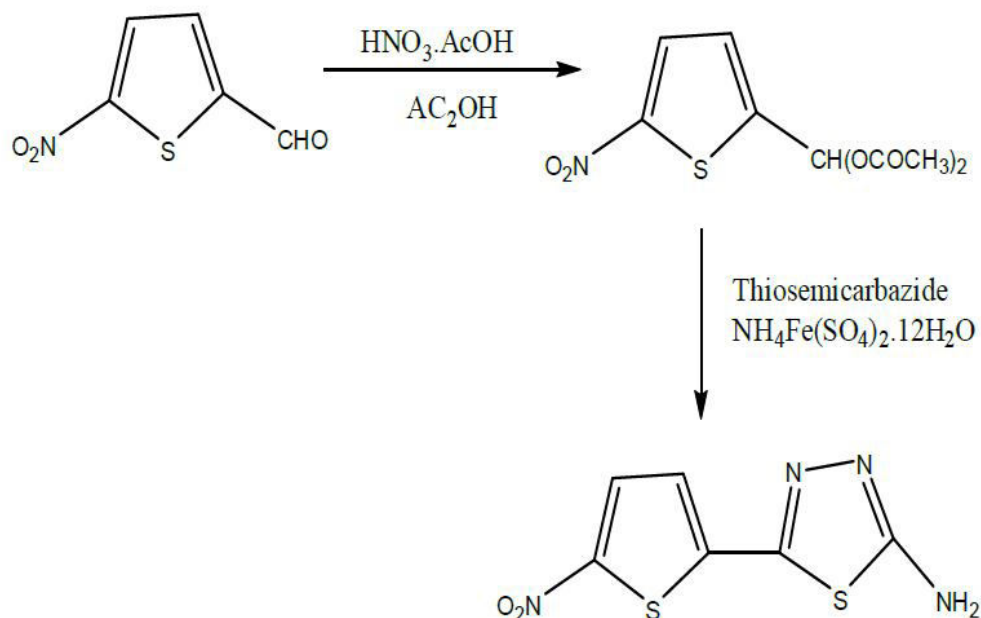
and anti-fouling in marine<sup>6</sup> Thiadiazole derivatives also possess fluorescence properties<sup>7</sup>.

Structure and aromatic properties: Bak et al. analyse the spectra of 1, 3, 4-thiadiazole and three isotopically substituted species. Using the analysis of difference between the measured bond lengths and covalent radii, it was concluded that the aromatic character, as measured by the  $\pi$ -electron delocalization decreases in the order –1, 2, 5-thiadiazole > thiophene > 1, 3, 4-thiadiazole<sup>8</sup> Dipole Moment: Bak et al. recorded the microwave spectra of 1, 3, 4-thiadiazole (I) and [34S] 1, 3, 4-thiadiazole (II) in the 15,000–30,000 Mc/sec region and measured the dipole moment of 1, 3, 4-thiadiazole in the gas phase by microwave technique and found a value of  $3.28 \pm 0.03$  D<sup>9</sup>.

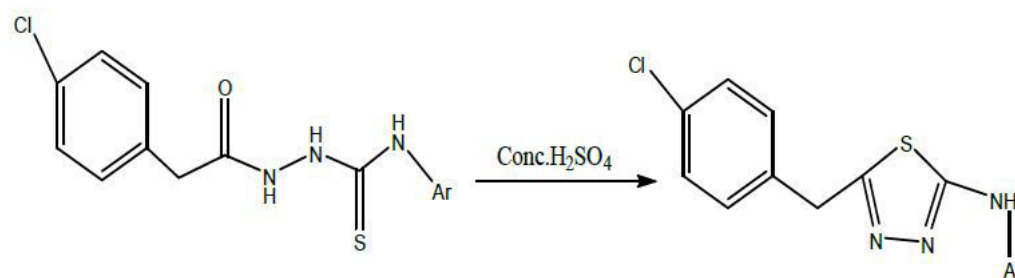
### **1.5. Method of synthesising thiadiazole**

#### **Synthesis of 1, 3, 4-thiadiazole**

**From thio-semicarbazide:** 1, 3, 4-thiadiazole can be synthesized by the cyclization of thiosemicarbazides<sup>10</sup> or ferric ammonium sulphate or ferric chloride catalysed oxidation cyclization of thiosemicarbazones<sup>11</sup>.

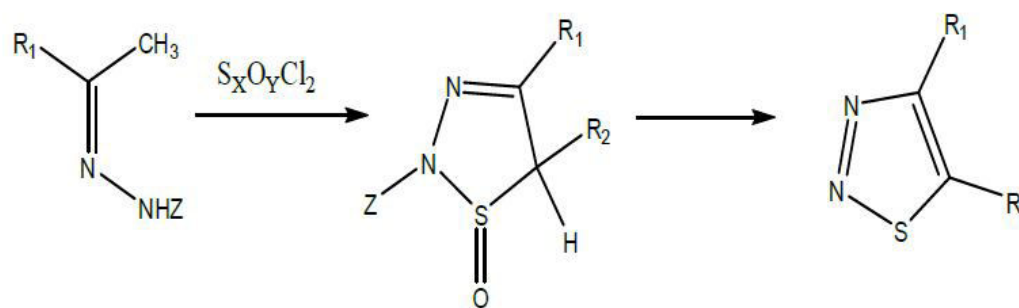


**From arylhydrazide:** Desai et al. synthesized various 2, 5-(4-chloro benzyl)-N-aryl-1, 3, 4- thiadiazole-2-amine by the cyclization of 2-(2-(4-chlorophenyl) acetyl)-N-aryl hydrazine carbothioamides with sulphuric acid<sup>12</sup>.

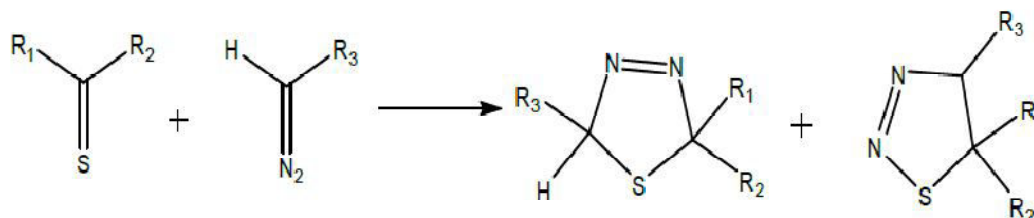


### Synthesis of 1, 2, 3-Thiadiazole

Cyclization of hydrazones with thionyl chloride (Hurd–Mori Synthesis):  
Hydrazone derivatives that are substituted at N-2 with an electron- withdrawing group ( $\text{Z} = \text{CONH}_2, \text{COOMe}, \text{COR}, \text{SO}_2\text{R}$ ) and possess an adjacent methylene group can cyclize in the presence of thionyl chloride to form 1, 2, 3-thiadiazoles.

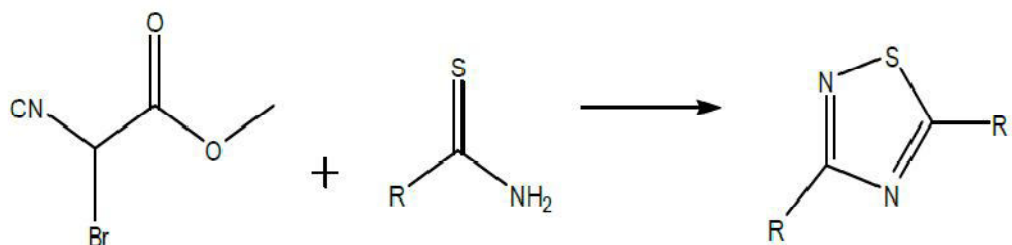


Cycloaddition of diazoalkanes onto a C=S bond (Pechmann Synthesis): When diazo compounds react with various thiocarbonyl compounds (thioketones, thioesters, thioamides, carbon disulfide, thioketenes, thiophosgene and isothiocyanates) 1, 2, 3-thiadiazoles are formed. The reaction of diazoalkanes with thioketones gives mixtures of 1, 3, 4-thiadiazolines and 1, 2, 3-thiadiazolines<sup>13-14</sup>.



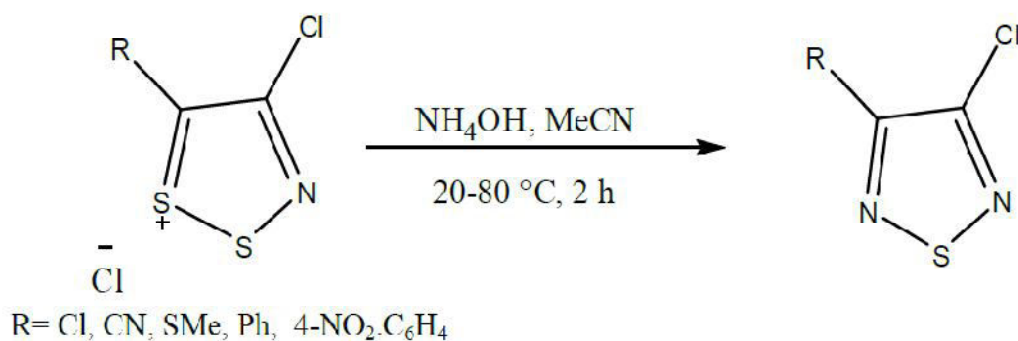
### Synthesis of 1, 2, 4-Thiadiazole

Condensation of aryl thioamides with methyl bromocyanoacetate: 1, 2, 4-thiadiazole can be synthesized when methyl bromocyanoacetate was allowed to react with aryl thioamide using different solvents. This reaction undergoes rapid condensation and provide quantitative yield<sup>15</sup>.



### Synthesis of 1, 2, 5-Thiadiazole

From acetonitrile: Treatment of an acetonitrile solution of the 1,2 3-dithiazoles with aqueous ammonia gave in three cases the corresponding 1,2,5-thiadiazoles<sup>16-19</sup>.



### 1.6. CANCER AND ANTI CANCER AGENTS

Today, the Greek term carcinoma is the medical term for a malignant tumour derived from epithelial cells. It is celsus who translated carcinos into the Latin cancer, also meaning crab. Galen used “oncos” to describe all tumours, the root for the modern word oncology.<sup>20, 21</sup>

Cancer is an important public health concern and in developed countries it represents the second leading cause of death, after cardiovascular disease. The resistance to chemotherapeutic antitumour agents by cancer cells could be

minimized using a combination of drugs with different and complementary mechanism of action. Therefore, there is a need to discover and develop useful new lead compounds of simple structure, exhibiting optimal *in vivo* antitumour potency and new mechanism of action.

**Cancer** is a disease in which a group of cells divides abnormally without any control, as to overrun and even destroy other tissues. These cells spread all over the body through the blood and lymph, giving rise to satellite lesions elsewhere and then eventually leading to death.

Cancer is one of the most wide spread and feared diseases in the western world today feared largely because it is known to be difficult to cure. The main reason for this difficulty is that cancer results from the uncontrolled multiplication of subtly modified normal human cells.

One of the main methods of modern cancer treatment is drug therapy (chemotherapy). Cancer is a major disease about one in four people will get it in some form during their life time, and at the present time about one in five of all death are due to cancer.

Currently there are three major ways of treating cancer:

- Radiation therapy
- Cytotoxic drugs.

Cancer arises from the mutation of a normal gene. Mutated genes that cause cancer are called **oncogenes**. A factor which brings about a mutation is called a

mutagen. Any agent that causes a cancer is called a **carcinogen** and is described as **carcinogenic**.

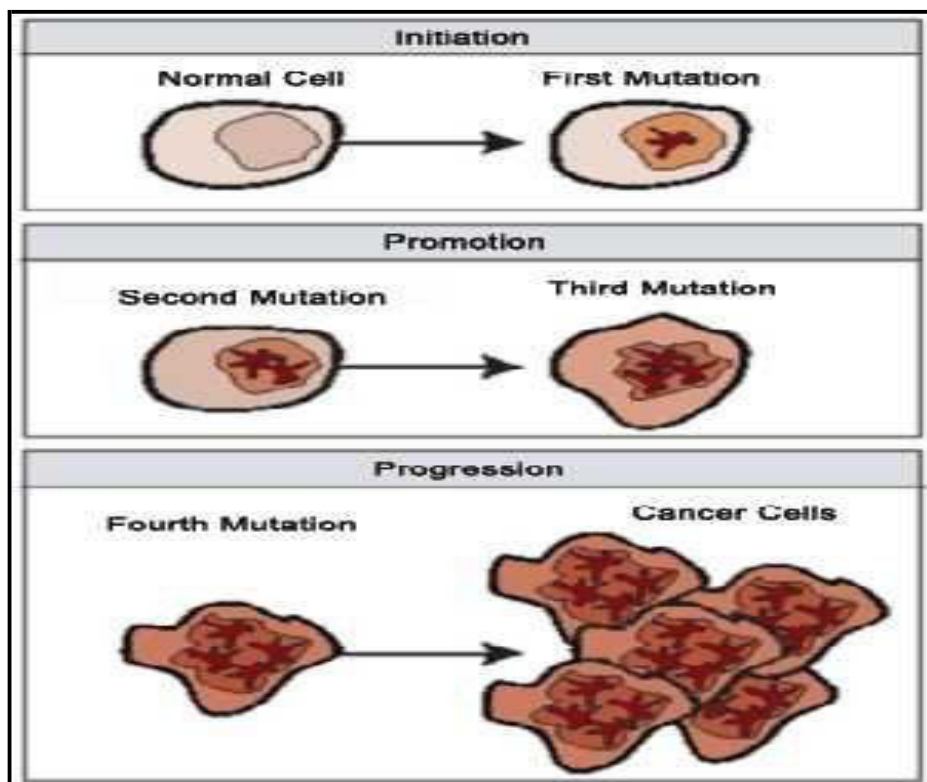


Fig.No.1 Types of cancer cell division



### **Types of tumour**

1. Benign tumours (do not spread from their site of origin, but can crowd out (squash ) surrounding cells e.g. brain tumour)
2. Malignant tumours (can spread from the original site and cause secondary tumours. this is called metastasis. They interfere with neighboring cells and can block blood vessels, the gut, glands, lungs etc.)

### **The Cell Cycle**

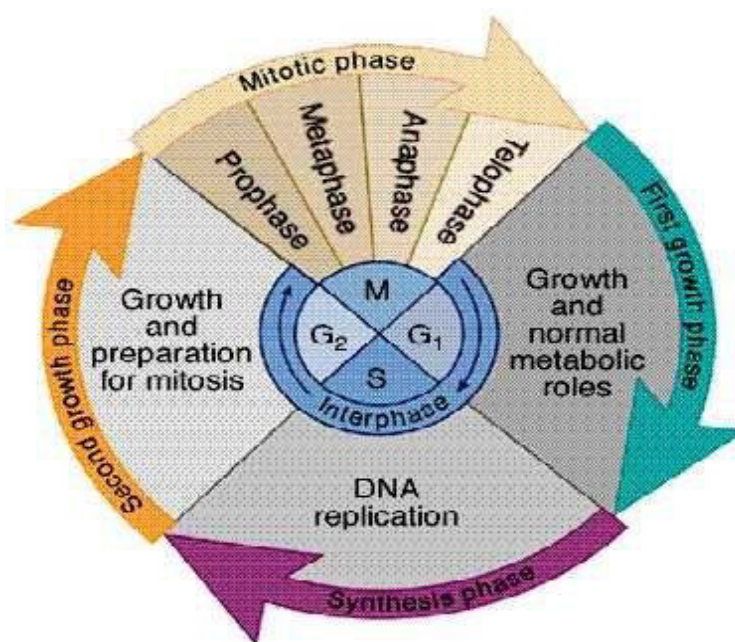


Fig.No.2 Phases of Cell cycle

- The cell cycle consists of four stages G<sub>1</sub>, S, G<sub>2</sub> and M.
- G<sub>1</sub> and G<sub>2</sub> are 'gap' phases in which the cell grows and prepares to divide.

- S is the synthesis phase in which the chromosomes (DNA) are copied (replicated).
- M is the mitotic phase in which the cell physically divides into two daughter cells.
- Most cells are NOT actively dividing. These cells are in a resting state (G).

### **Mitosis (M phase)**

- Mitosis in normal cells produces two cells with identical genetic content.
- Mitosis has four sub-phases.
- Prophase - Chromosomes condense, the nuclear membrane breaks down and spindle fibers form
- Metaphase - The replicated chromosomes line up in the middle of the cell.
- Anaphase - Chromosomes separate and the cell becomes elongated with distinct ends (poles)
- Telophase - Nuclear envelopes reform at the two poles and new cell membranes are formed to create two independent cells
- Cytotoxicity is the cell killing property of a chemical compounds .cell death can occur by either of two distant mechanism, necrosis or apoptosis.
- Necrosis is physical or chemical damage, where apoptosis is the physiological process by which unwanted cells are eliminated during development and other normal biological processes.

**Types of cancer**

Lung Cancer	Lung
Breast Cancer	Breast
Cervical Cancer	Cervical
Colon Cancer	Colon
Leukemia	Blood
Testicular Cancer	Testis
Brain Cancer	Brain
Kidney Cancer	Kidney
Thyroid Cancer	Thyroid Gland
Liver Cancer	Liver
Bone Cancer	Bone marrow

Table No.1 Types of Cancer

**Causes of cancer**

- DNA Mutations
  1. Radiation – other environmental (tobacco, alcohol, radon, asbestos, chemicals etc).
  2. Random somatic mutations
  3. Inherited germ line mutations
- Genetic predisposition –
  1. Rb, p53, APC, CDKN2A, BRCA1, BRCA2
- Infectious agents
  1. Viral

A. HPV(Human papilloma virus) – cervical cancer

B. Hepatitis – liver cancer

2. Bacterial

A. *H. pylori* – stomach cancer

### **Diagnosis of cancer**

- Biopsy of the tumor
- Blood tests (which look for chemicals such as tumor makers)
- Bone marrow biopsy (for lymphoma or leukemia)
- Chest x-ray
- Complete blood count
- CT scan & MRI scan

### **Treatment of Cancer Traditional treatment**

- 1.Surgery: it is the first treatment which is used to removed solid tumors, and for early stage cancer and benign tumors.
- 2.Radiation: it kills the cancer cells with high energy rays targeted to the tumor. It acts by damaging DNA and preventing its replication.

### **Newer treatment**

1. Hormone therapy: by using Hormones and anti – Hormone.

2. Chemotherapy: Chemotherapy means treatment with anti cancer drugs and they are given to destroy or control cancer cells.

### **1.7 CERVICAL CANCER**

The American Cancer Society's most recent estimates for cervical cancer in the United States are for 2011:

About 12,710 new cases of invasive cervical cancer will be diagnosed per year.

- About 4,290 women will die from cervical cancer per year.

Cervical cancer is one of the most common types of cancer and a majority mortality factor of women worldwide.

The cervix is the lower part of the uterus (womb). The part of the cervix closest to the body the uterus is called the endocervix. The part next to the vagina is the exocervix. The two main types of cells covering the cervix are squamous cells (on the endocervix). The place where these two cell types meet is called the transformation zone. Most cervical cancers start in the transformation zone.

Normally, cervical cells grow in an orderly fashion. However, when control of that growth is lost, cells divide too frequently and too fast. Nearly all cervical cancers arise of the inner of the cervix.

**There are several types of cervical cancer:**

**Squamous cell carcinoma (SCC)** is the most common type of cervical cancer, accounting for 85% to 90% of all cases. It develops from the cells that line the inner part of the cervix, called the squamous cells.

**Adenocarcinoma** develops from the column shaped cells that line the mucous producing glands of the cervix. In rare instances, adenocarcinoma accounts for about 10% of all cervical cancers.

**Mixed carcinomas (adenosquamous carcinomas)** combine features of both squamous cell carcinoma and adenocarcinoma.

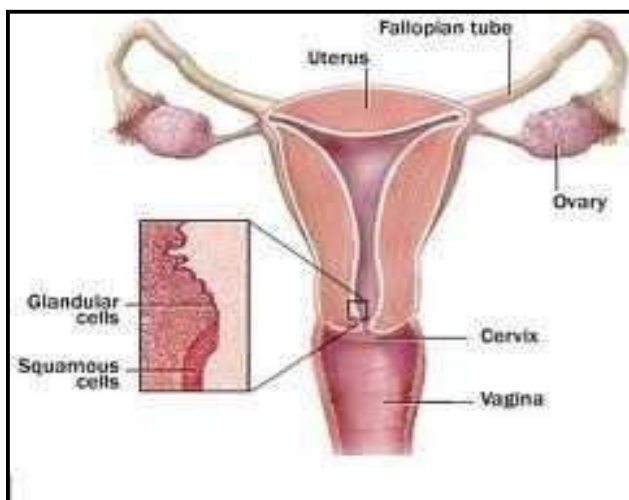


Fig.No.3 Cervix

### **Treatment of Cervical Cancer**

- Surgery
  - Pre invasive cervical cancer
  - Cryosurgery
  - Laser surgery
  - Conization
- ❖ Invasive cervical cancer
  1. Simple hysterectomy – removal of the body of the uterus and cervix
  2. Radical hysterectomy and pelvic lymph node dissection Removal of entire uterus, surrounding tissue, upper part of the vagina and lymph nodes from the cervix.
- Radiation
- Chemotherapy

The drugs used to combat cancer belong to one of the two broad categories. The first is cytotoxic drugs (cell killing) and the second is cytostatic drugs (cell stabilizing). Both the categories lead to a reduction in the size of tumor because cancer cells (for various reasons) have such a high mortality rate that simply preventing them from dividing will lead to a reduction in the population.

The majority of drugs used for treatment of cancer today are cytotoxic (cell killing) drugs that work by interfering in some way with the operation of the cell's DNA. Cytotoxic drugs have the potential to be very harmful to the body unless they are very specific to cancer cells something difficult to achieve because the

modifications that change a healthy cell into a cancerous one are very subtle. A major challenge is to design new drugs that will be more selective for cancer cells, and thus have lesser side effects.

The development of a new pharmaceutical is a complex process, but can be broken down to three main steps:

- Discovery of a new potentially use full molecule.
- Appropriate molecular modification to produce a molecule with the best combination of properties.
- Development of this molecule into a safe and affordable drug.



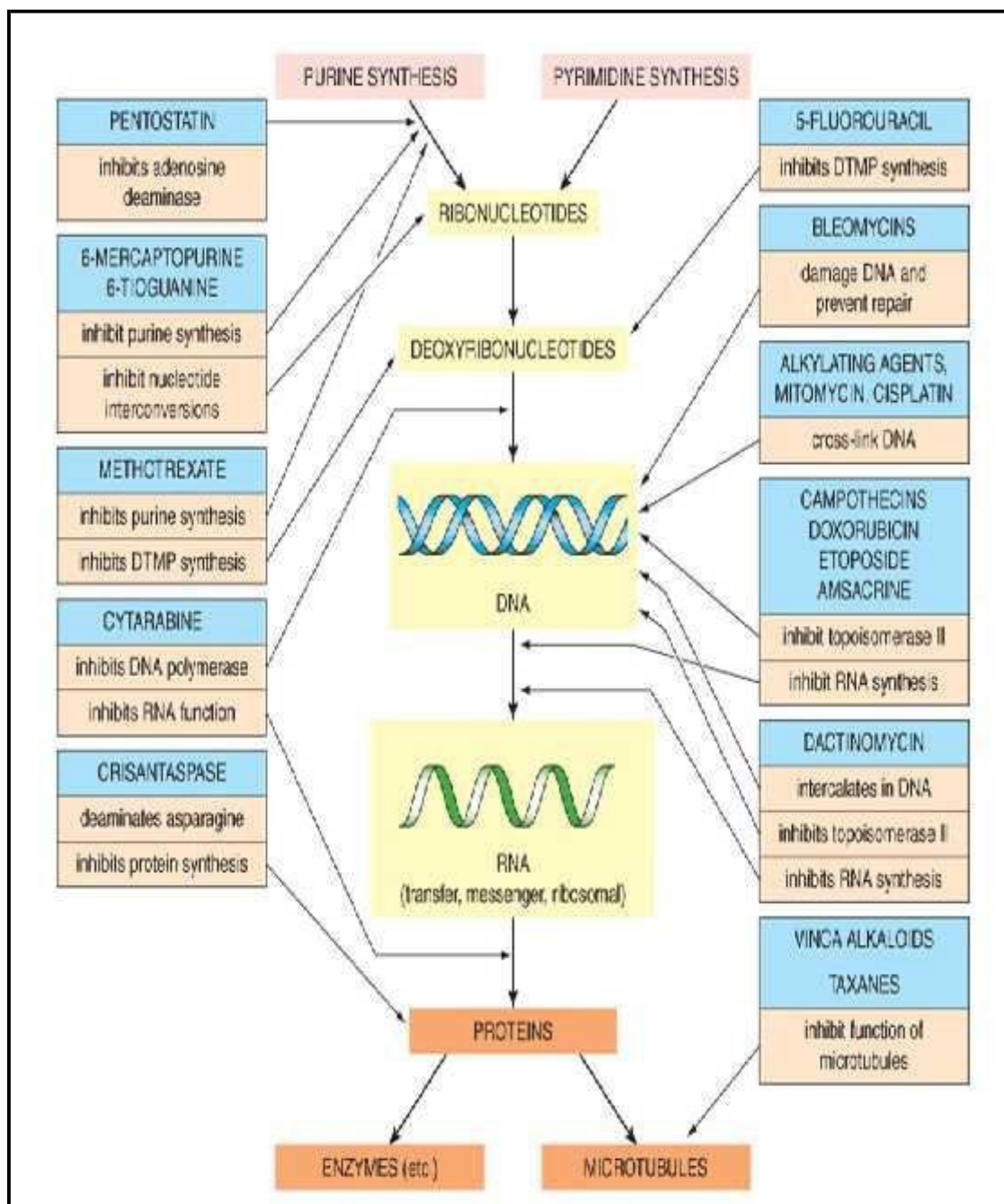


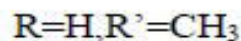
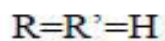
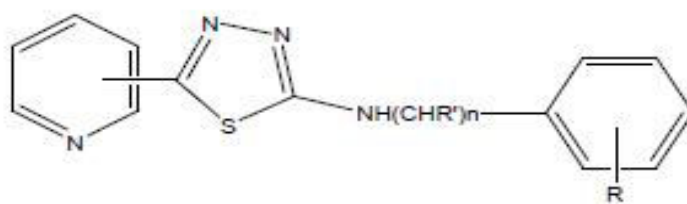
Fig.No.4 MOA of Cytotoxic drugs

## CHAPTER -2

### REVIEW OF LITERATURE

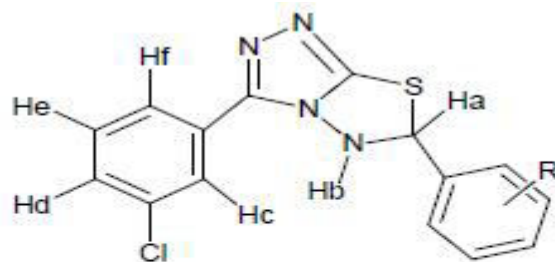
#### ANTIMICROBIAL

Zamani et al<sup>22</sup> (2004) has reported new 2, 5-disubstituted derivatives of 1, 3, 4-thiadiazoles (**1**) containing isomeric pyridyl were obtained from cyclization of corresponding thiosemicarbazides under acidic conditions. Most of the synthesized compounds have been found to be active against both gram-positive and gram-negative bacteria at less than 3.6 mg/ml. The compound is most active against all seventeen used gram-positive and gram-negative bacteria.



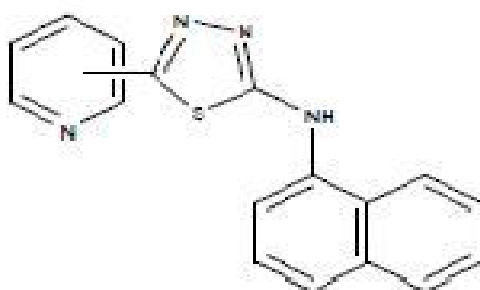
(1)

Purohit et al<sup>23</sup> (2011) has reported the synthesis of fused ring system 3-(3-chlorophenyl)-6-aryl-5,6-dihydro[1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles (**2**) reaction of 4-amino-5-(3 chlorophenyl)-4H-1,2,4-triazole-3-thiol. All the newly synthesized compounds were screened for their antimicrobial activity. Some of the compounds exhibited significant inhibition on bacterial and fungal growth as compared to standard drugs.



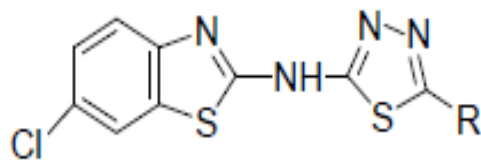
(2)

Zamani et al<sup>24</sup>. (2003) has synthesized a new 1, 2, 4-tri and 1, 3, 4-thiadiazoles (**3**) bearing isomeric pyridyl and 1- naphthyl using 1,4-disubstituted thiosemicarbazides in alkaline and acidic media, respectively. The antibacterial studies of some of the synthesized compounds against *S. aureus* and *E. coli* as MIC values are reported. None of them have important antibacterial activities.



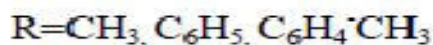
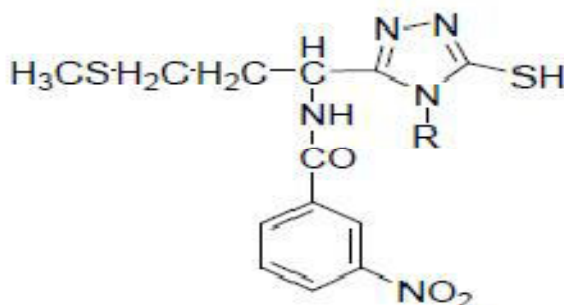
(3)

Amir et al<sup>25</sup>. (2009) has reported the synthesis of thiosemicardazide of 6-chloro-2-aminobenzotiazole (**4**) on cyclization with different carboxylic acid in  $POCl_3$  and substituted azalactones in pyridine provide the corresponding 2-aryl-5-(6chloro-1,3benzothiazole-2-yl-amino)- 1,3,4-thiadiazoles. All the compounds have been evaluated in vitro for their antimicrobial activities against several microbes and show significant activity.



(4)

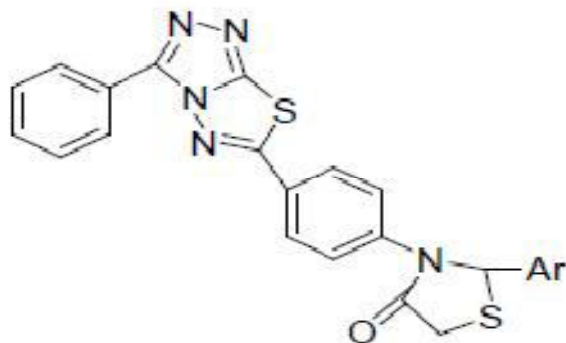
Otilia Pintilie and co-workers<sup>26</sup> (2007) has reported the new synthesis that is 1,3,4-thiadiazole(5) and 1,2,4-triazole compounds containing a D,L-methionine moiety (5) were synthesized by intramolecular cyclization of 1,4-disubstituted thiosemicarbazides in acid and alkaline media, respectively.



(5)

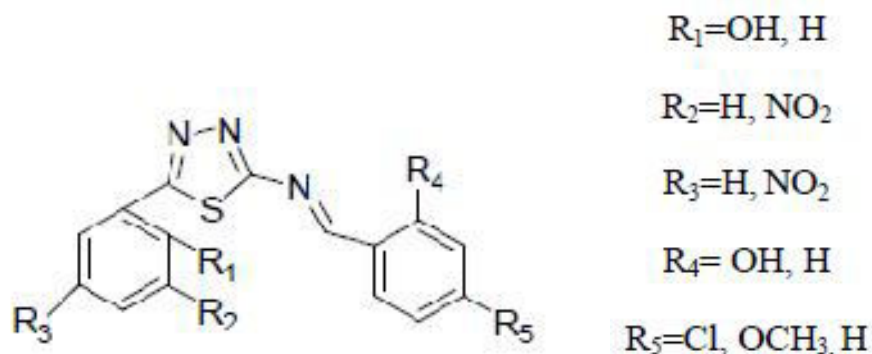
## ANTIHELMINTHICS

Parmar Kokila and co-workers<sup>27</sup> (2011) has prepared a new and biologically active [1,2,4] triazolo [3,4-b][1,3,4] thiadiazole-2-aryl-thiazolidinone-4-ones (6) by reaction of Schiff bases with mercapto acetic acid in presence of THF with adding anhydrous  $\text{ZnCl}_2$ . The compounds have been evaluated for antibacterial activity against *B. subtilis*, *S. aureus*, *P. aeruginosa*.



(6)

Mathew et al<sup>28</sup> (2010) has synthesized some Schiff bases of 5-phenyl substituted, 2-amino 1, 3, 4 thiadiazole derivatives (7). This reaction between various aryl carboxylic acids with thiosemicarbazide in presence of dehydrating agent like Conc. H<sub>2</sub>SO<sub>4</sub> to form 5-phenyl substituted, 2-amino 1, 3, 4 thiadiazole derivatives. These derivatives on further treatment with various aldehydes to form Schiff base.

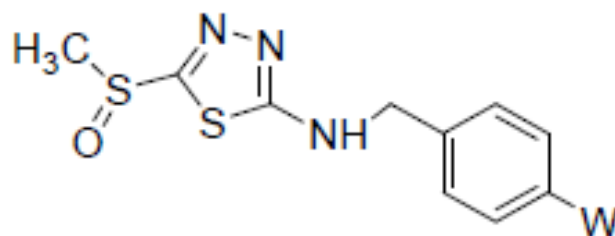


(7)

## ANTI INFLAMMATORY

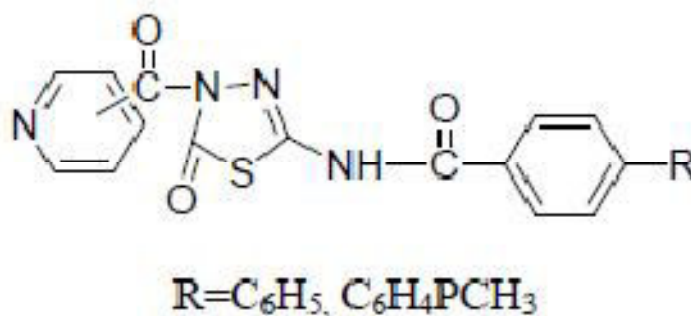
Varandas et al<sup>29</sup>, (2005) has reported design, synthesis and evaluation of the anti-inflammatory, analgesic, and antiplatelet properties of new 1,3,4-thiadiazole derivatives (8), structurally planned by exploiting the molecular hybridization approach between diuretic drug acetazolamide and a 1,3-benzodioxole COX-2 inhibitor, previously developed. The *in vivo* pharmacological evaluation of these new compounds

lead us to identify the para-fluoro-substituted derivative 8b as a new prototype, more active than celecoxib at the same molar concentration.



(8)

Schenone et al.<sup>30</sup> (2001) has prepared the series of 3-arylsulphonyl-5-arylamino-1,3,4-thiadiazol-2(3H)ones (**9**) with potential anti-inflammatory and analgesic activity. Pharmacological results revealed that all the title compounds, endowed with an arylsulphonyl side chain, possess good analgesic activity and fair anti-inflammatory properties. The analgesic profile of the two series, evaluated by the acetic acid writhing test, showed that compounds 2c, 2f and 2h, in particular, were the most active.



(9)

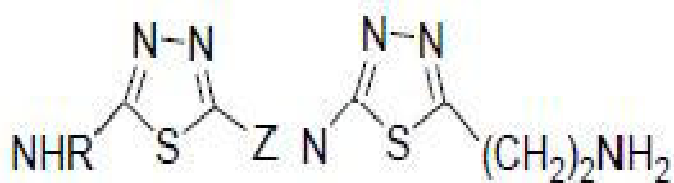
Amir and co-workers<sup>31</sup> (2007) has reported various 1,3,4-oxadiazoles, 1,2,4-triazoles, 1,3,4-thiadiazoles, and 1,2,4-triazine derivatives of ibuprofen by cyclization of 2-(4-butyl phenyl) propionic acid hydrazide and N1-[2-(4-i-butylphenyl)-propionyl]-N4-alkyl/aryl-thiosemicarbazides under various reaction conditions. The cyclized

derivatives were screened for their anti-inflammatory activity by the carrageenan induced rat paw edema method and showed 50 to 86% inhibition, whereas the standard drug ibuprofen showed 92% inhibition at the same oral dose.

Varandas et al<sup>32</sup> (2010) has reported a design, synthesis and evaluation of the anti-inflammatory, analgesic, and antiplatelet properties of new 1,3,4-thiadiazole derivatives, structurally planned by exploiting the molecular hybridization approach between diuretic drug acetazolamide and a 1,3-benzodioxole COX-2 inhibitor, previously developed. The in vivo pharmacological evaluation of these new compounds lead us to identify the para-fluoro-substituted derivative 8b as a new prototype, more active than celecoxib at the same molar concentration.

## ANTIVIRAL

Chen et al<sup>33</sup> (2010) has reported a synthesis of new 5-(4-chlorophenyl)-N-substituted-N-1,3,4-thiadiazole-2-sulfonamide derivatives in six-steps. Esterification of 4-chlorobenzoic acid (**10**) with methanol and subsequent hydrazination, salt formation and cyclization afforded 5-(4-chlorophenyl)-1,3,4-thiadiazole-2-thiol. Conversion of this intermediate into sulfonyl chloride, followed by nucleophilic attack of the amines gave the title sulfonamides.



(10)

Friedrick and Hayden et al<sup>34</sup> (1994) has reported the efficacy and safety of oral LY217896 for prevention of experimental influenza A/Kawasaki/86 (H1N1) virus infection were assessed in susceptible males randomly assigned to receive LY217896 (75 mg) or placebo once daily for 7 days beginning 24 h prior to viral challenge. The rates of virus shedding (100% in both groups), days of viral shedding ( $3.1 \pm 1.3$  for the LY217896 group;  $2.8 \pm 1.3$  for the placebo group), and titers of virus in nasal washings did not differ between the groups. Mild upper respiratory tract illness (72% in the LY217896 group; 69% in the placebo group) developed in similar proportions of each group. LY217896 was associated with asymptomatic rises in serum uric acid levels and was ineffective in modifying the virologic or clinical course of experimental influenza A (H1N1) virus infection.

Jones et al<sup>35</sup> (2009) has prepared Isosorbide-2-aspirinate-5-salicylate is a true aspirin prodrug in human blood because it can be effectively hydrolyzed to aspirin upon interaction with plasma BuChE. It shows that the identity of the remote 5-ester dictates whether aspirin is among the products of plasma-mediated hydrolysis. By observing the requirements for aspirin release from an initial panel of isosorbide-based esters, it is able to introduce nitro oxy methyl groups at the 5-position while maintaining ability to release aspirin. Several of these compounds are potent inhibitors of platelet aggregation. The design of these compounds will allow better exploration of cross-talk between COX inhibition and nitric oxide release and potentially lead to the development of selective COX-1 acetylating drugs without gastric toxicity.

Bonina et al<sup>36</sup> (1982) has reported preparation of 2-amino-5-(2-sulfamoylphenyl)-1,3,4-thiadiazole(11) (G413) was shown to possess high activity



against DNA viruses (herpes simplex viruses 1 and 2 and adenovirus 17 and RNA viruses (poliovirus 1, echovirus 2, and coxsackievirus B4) experiments on the replicative cycle of poliovirus 1 and production of infectious RNA viruses demonstrate that this compound probably prevents assembly of virus particles by acting on structural proteins. In the present experiments, results concerning the activity of derivatives of G-413 after side-chain modification are reported. Modification of the primary amine H to CH<sub>3</sub> or CH<sub>2</sub>-CH=CH<sub>2</sub> produced a loss of activity against DNA viruses, but inhibitory action on RNA viruses was preserved. Modification to CH<sub>2</sub>CH<sub>3</sub> resulted in the loss of antiviral activity.

## ANTICANCER

Şerban and coworkers<sup>37</sup> (2010) has reported some 2-R-5-formyl-1,3,4-thiadiazole derivatives (**11**) have been synthesized and characterized by their spectral data through Sommelet reaction, of some hexamethylenetetramine salts from which some new heterocyclic aldehydes resulted.

Ilango et al<sup>38</sup> (2010) has prepared a facile synthesis of 3, 6-disubstituted- 1, 2, 4-triazolo- [3, 4-b] -1, 3, 4- thiadiazoles) by condensing 3-aryl substituted 4-amino-5-mercapto (4H)-1, 2, 4-triazole with various aromatic acids.



(11)

Sahu and co-workers<sup>39</sup> (2013) has reported the substituted salicylic acid and thiosemicarbazide were refluxed in acidic medium to obtain 2-amino-5-(o-hydroxysubstituted phenyl)-1, 3, 4-thiadiazol which on treatment with various aryl aldehydes and then with thioglycolic acid gives 2-(substitutedphenyl)-3-(2-hydroxysubstitutedphenyl-1,3,4-thiadiazol-2yl) thiazolidin-4-ones. Structures of the compounds (3a-l) were confirmed on the basis of IR and <sup>1</sup>HNMR data. All the compounds were tested against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger*.

**CHAPTER -3****RESEARCH ENVISAGED****3.1 Objective of the Present Work**

1, 3, 4-thiadiazole possess a wide spectrum of biological and pharmacological activity due to the presence of nitrogen and sulfur axis which is considered to be responsible for the structural features to impart their activities

Despite the optimal use of available anti-cancer drugs (ACDs), many patients with cancer fail to experience neoplastic control and others do so only at the expense of significant toxic side effects. The limitations with the conventional ACDs highlighted the need for developing newer agents to treat cancer and therefore new, less toxic and more effective drugs are required. Based on the bioisostere concept in the present study we explored 1, 3, 4-thiadiazole as a pharmacophore for the development of new anticancer drugs.

1, 3, 4-Thiadiazoles are five membered ring system containing sulphur and nitrogen atom and received much attention of medicinal chemists due to their potential biological activities. Various substituents at C-2 and C-5 of thiadiazoles results in potent anticancer activity. Prompted by these reports, we aimed to prepare the following series of 2, 5-disubstituted-1, 3, 4-thiadiazole derivatives as potent anti-cancer agents.

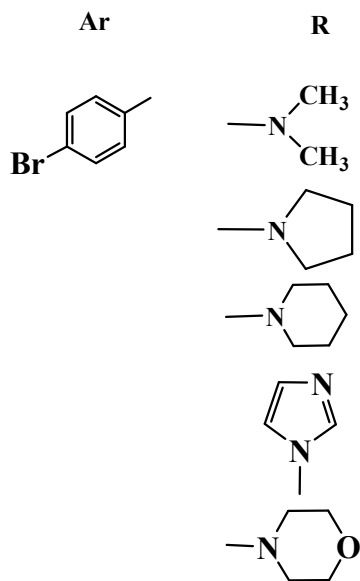
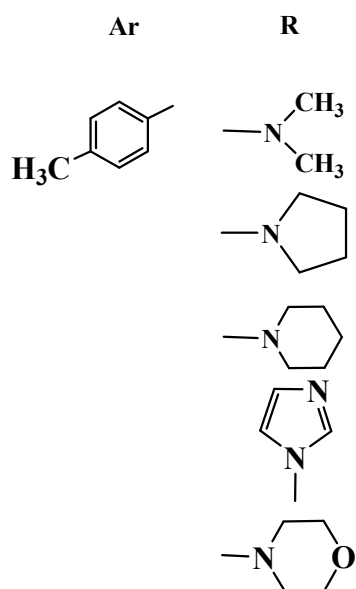
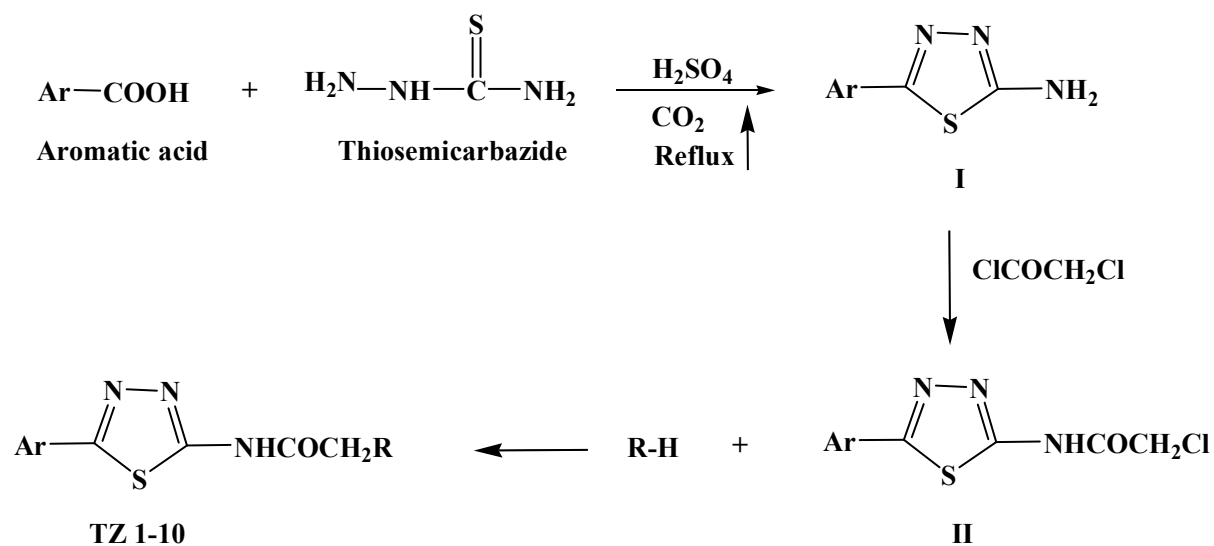
**Hence the specific aims & objectives of the present study are,**

- To synthesize a series of novel 2, 5-disubstituted thiadiazoles.
- To characterize the synthesized compounds by IR, NMR, Mass spectra and elemental analysis.

- To evaluate the test compounds for *in vitro* anti-cancer activity by
  - MTT *in vitro* assay method

The title compounds are planned to synthesize by using the following synthetic routes mentioned in the following Schemes.

## SCHEME



## CHAPTER – 4

### 4.1 EXPERIMENTAL WORK

#### 4.1.1 MATERIALS AND METHODS

Melting points (mp) were taken in open capillaries on Thomas Hoover melting point apparatus and are uncorrected. The IR spectra were recorded in film or in potassium bromide disks on a Perkin-Elmer 398 spectrometer. The  $^1\text{H}$  spectra were recorded on a DPX-500 MHz Bruker FT-NMR spectrometer. The chemical shifts were reported as parts per million ( $\delta$  ppm) tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained on a JEOL-SX-102 instrument using fast atom bombardment (FAB positive). Elemental analysis was performed on a Perkin-Elmer 2400 C, H, N analyzer and values were within the acceptable limits of the calculated values. The progress of the reaction was monitored on readymade silica gel plates (Merck) using chloroform-methanol (9:1) as a solvent system. Iodine was used as a developing agent. Spectral data (IR, NMR and mass spectra) confirmed the structures of the synthesized compounds and the purity of these compounds was ascertained by microanalysis. Elemental (C,H,N) analysis indicated that the calculated and observed values were within the acceptable limits ( $\pm 0.4\%$ ). All chemicals and reagents were obtained from Aldrich (USA), Lancaster (UK) or Spectrochem Pvt.Ltd (India) and were used without further purification.

**4.1.1.1. General Procedure for synthesis of 5-aryl -1, 3, 4-thiadiazol-2-amine (I).**

A mixture of thiosemicarbazide (0.1mol) and aryl carboxylic acid (0.1mol) in conc.sulphuric acid (10 drops) was refluxed for 1 hr & poured onto crushed ice. The solid separated out was filtered, washed with water & recrystallized from ethanol.

**4.1.1.2. General Procedure for synthesis of substituted N-(5-aryl-1, 3,4-thiadiazole-2-yl)-2-chloroacetamide (II)**

Substituted amino compounds (0.3mol) were dissolved in glacial acetic acid (20ml) containing 20ml of saturated solution of sodium acetate. In case the substance did not dissolve completely, the mixture was warmed and the solution was cooled in ice bath with stirring. To this chloroacetyl chloride was added drop wise (0.06mol) with stirring. After half an hour white product separated and filtered. The product was washed with 50% aqueous acetic acid and finally with water. It was purified by recrystallization from ethyl alcohol.

**4.1.1.3. General Procedure for synthesis of substituted N-(5-aryl-1, 3, 4-thiadiazole-2-yl)-2-alkyl / aryl substituted acetamides (TZ 1-10)**

A mixture of N-(5 aryl-1, 3, 4-thiadiazole-2-yl)-2-chloroacetamide (0.01mol) is taken in 25ml of ethyl alcohol and alkyl and aryl substituted derivatives (0.01mol) were added and refluxed for 4hr. The resulting mixture was purified by recrystallization from alcohol.

**4.1.2. Synthesis of 5-p-tolyl-1, 3, 4-thiadiazol-2-amine (I)**

Yield	: 2.46 g; 91.0 %
Melting Point	: 156-159 °C
Rf Value	: 0.86 (benzene: ethyl acetate (8:2))
Molecular Formula	: C <sub>9</sub> H <sub>9</sub> N <sub>3</sub> S
Molecular Weight	: 191(M <sup>+</sup> )
IR (KBr) cm <sup>-1</sup>	: 3290 (NH <sub>2</sub> ), 3045 (Ar-CH), 1620 (C=N Str), 675 (C-S-C).
<sup>1</sup> H NMR (CDCl <sub>3</sub> ) δ ppm	: <sup>1</sup> H NMR (CDCl <sub>3</sub> ) δ (ppm): 2.35 (s, 3H, CH <sub>3</sub> ), 4.02(s, 2H, NH <sub>2</sub> ), 7.12 (d, <i>J</i> = 8.0 Hz, 2H, Ar-H), 7.36 (d, <i>J</i> = 8.0Hz, 2H, Ar-H).
Elemental Analysis	
Calculated	: C, 56.52; H, 4.74; N, 21.97.
Found	: C, 56.50; H, 4.73; N, 21.95.



**4.1.3. Synthesis of 5-(4-bromophenyl)-1,3,4-thiadiazol-2-amine (Ia)**

Yield : 2.57 g; 76.0 %

Melting Point : 197-199 °C

Rf Value : 0.74 (benzene: ethyl acetate (8:2))

Molecular Formula : C<sub>8</sub>H<sub>6</sub>BrN<sub>3</sub>S

Molecular Weight : 256(M<sup>+</sup>), 258 (M+2)

IR (KBr) cm<sup>-1</sup> : 3290 (NH<sub>2</sub>), 3045 (Ar-CH), 1620 (C=N Str), 675 (C-S-C), 651 (C-Br).

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ ppm : <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ (ppm): 4.12 (s, 2H, NH<sub>2</sub>), 7.37 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.49 (d, *J* = 8.0 Hz, 2H, Ar-H).

Elemental Analysis

Calculated : C, 37.52; H, 2.36 ; N, 16.41.

Found : C, 37.51; H, 2.34; N, 16.40.

**4.1.4. Synthesis of 2-chloro-N-(5-methyl-1,3,4-thiadiazol-2-yl)acetamide (II).**

Yield : 2.10 g; 69.0 %

Melting Point : 142-145 °C

Rf Value : 0.72 (benzene: ethyl acetate (8:2))

Molecular Formula : C<sub>5</sub>H<sub>6</sub>ClN<sub>3</sub>OS

Molecular Weight : 191(M<sup>+</sup>)

IR (KBr) cm<sup>-1</sup> : 3426 (NH), 3040 (Ar-CH), 1710(C=O),1617 (C=N Str), 674 (C-S-C).

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ ppm : <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ (ppm): 2.37(s, 3H, CH<sub>3</sub>), 4.27 (s, 2H, CH<sub>2</sub>), 7.12 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.36 (d, *J* = 8.0Hz, 2H, Ar-H), 8.02 (s, 1H, NH).

Elemental Analysis

Calculated : C, 31.34; H, 3.16 N, 21.93.

Found : C, 31.31; H, 3.15 N, 21.90.

**4.1.5. Synthesis of N-(5-bromo-1, 3, 4-thiadiazol-2-yl) - 2-chloroacetamide (IIa)**

Yield	: 2.38 g; 79.0 %
Melting Point	: 208-210 °C
Rf Value	: 0.68 (benzene: ethyl acetate (8:2))
Molecular Formula	: C <sub>4</sub> H <sub>3</sub> BrClN <sub>3</sub> OS
Molecular Weight	: 256(M <sup>+</sup> );258(M+2)
IR (KBr) cm <sup>-1</sup>	: 3432 (NH), 3058 (Ar-CH), 1708(C=O),1626 (C=N Str), 683 (C-S-C), 631(C-Br).
<sup>1</sup> H NMR (CDCl <sub>3</sub> ) δ ppm	: <sup>1</sup> H NMR (CDCl <sub>3</sub> ) δ (ppm): 4.28 (s, 2H, CH <sub>2</sub> ), 7.35 (d, <i>J</i> = 8.0 Hz, 2H, Ar-H), 7.45 (d, <i>J</i> = 8.0Hz, 2H, Ar-H). 8.0 (s, 1H, NH).
Elemental Analysis	
Calculated	: C, 18.73; H, 1.18; N, 16.38.
Found	: C, 18.71; H, 1.17; N, 16.35.

**4.1.5. Synthesis of 2-(dimethylamino)-N-(5-p-tolyl-1,3,4-thiadiazol-2-yl)acetamide(TZ1) .**

Yield	: 1.92 g; 70.2 %
Melting Point	: 150-153 °C
Rf Value	: 0.73 (benzene: ethyl acetate (8:2))
Molecular Formula	: C <sub>13</sub> H <sub>16</sub> N <sub>4</sub> OS
Molecular Weight	: 276(M <sup>+</sup> )
IR (KBr) cm <sup>-1</sup>	:3457(NH),3056(Ar-CH),2929(N(CH <sub>3</sub> ) <sub>2</sub> ), 1716(C=O),1622 (C=Nstr), 651 (C-S-C).
<sup>1</sup> H NMR (CDCl <sub>3</sub> ) δ ppm	: <sup>1</sup> H NMR (CDCl <sub>3</sub> ) δ (ppm): 2.27 ( d, 3H, CH <sub>3</sub> ), 2.35(s, 3H, CH <sub>3</sub> ), 3.25 (s, 2H,CH <sub>2</sub> ), 7.14(d, <i>J</i> = 8.0 Hz,2H, Ar-H), 7.34 (d, <i>J</i> =8.0Hz,2H, ArH), 8.06 (s,1H,NH).
Elemental Analysis	
Calculated	: C, 56.50; H, 5.84; N, 20.27.
Found	: C, 56.48; H, 5.84; N, 20.25.

**4.1.6. Synthesis of 2-(pyrrolidino)-N-(5-p-tolyl-1, 3, 4 -thiadiazol-2-yl) acetamide(TZ 2).**

Yield : 1.88 g; 67.4 %

Melting Point : 184-186 °C

Rf Value : 0.77 (benzene: ethyl acetate (8:2))

Molecular Formula : C<sub>15</sub>H<sub>18</sub>N<sub>4</sub>OS

Molecular Weight : 302(M<sup>+</sup>)

IR(KBr)cm<sup>-1</sup> : 3478(NH), 3042(Ar-CH), 2926(CH<sub>2</sub>),  
1704(C=O), 1647 (C=Nstr), 693 (C-S-C).

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ ppm : <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ (ppm): 1.59 (d, 2H, CH<sub>2</sub>), 2.25 (d, 2H, CH<sub>2</sub>), 2.32(s, 3H, CH<sub>3</sub>), 3.24(s, 2H, CH<sub>2</sub>), 7.14 (d, *J*= 7.5 Hz, 2H, Ar-H), 7.38(d, *J* = 8.0 Hz, 2H, Ar-H), 8.06(s, 1H, NH).

**Elemental Analysis**

Calculated : C, 59.58; H, 6.00; N, 18.51.

Found : C, 59.56; H, 5.99; N, 18.53.

**4.1.7. Synthesis of 2-(piperidino)-N-(5-p-tolyl-1, 3, 4 -thiadiazol-2-yl) acetamide (TZ 3).**

Yield	: 1.78 g; 62.4 %
Melting Point	: 216-218 °C
Rf Value	: 0.82 (benzene: ethyl acetate (8:2))
Molecular Formula	: C <sub>16</sub> H <sub>20</sub> N <sub>4</sub> OS
Molecular Weight	: 316 (M <sup>+</sup> )
IR (KBr) cm <sup>-1</sup>	: 3418 (NH), 3057 (Ar-CH), 2929(CH <sub>3</sub> ), 1714(C=O), 1629 (C=N Str), 689 (C-S-C),
<sup>1</sup> H NMR (CDCl <sub>3</sub> ) δ ppm	: <sup>1</sup> H NMR (CDCl <sub>3</sub> ) δ (ppm): 1.50 (d, 2H, CH <sub>2</sub> ), 2.24 (s, 2H, CH <sub>2</sub> ), 2.25 (s, 2H, CH <sub>2</sub> ) 2.35(s, 3H, CH <sub>3</sub> ), 3.27(s, 2H, CH <sub>2</sub> ), 7.10(m, <i>J</i> = 7.5 Hz, 4H, Ar-H), 7.66(m, <i>J</i> = 8.0 Hz, 8H, Ar- H), 8.01(s, 1H, NH).
Elemental Analysis	
Calculated	: C, 60.73; H, 6.37; N, 17.71.
Found	: C, 60.0; H, 6.36; N, 17.69.

**4.1.8. Synthesis of 2-(imidazolo)-N-(5-p-tolyl-1, 3, 4-thiadiazol-2-yl) acetamide (TZ 4).**

Yield : 1.68 g; 66.4 %

Melting Point : 190-192 °C

Rf Value : 0.69 (benzene: ethyl acetate (8:2))

Molecular Formula : C<sub>14</sub>H<sub>13</sub>N<sub>5</sub>OS

Molecular Weight : 299 (M<sup>+</sup>)

IR (KBr) cm<sup>-1</sup> : 3417(NH), 3058 (Ar-CH), 2929(CH<sub>3</sub>), 1710(C=O),  
1626 (C=N Str), 689 (C-S-C).

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ ppm : <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ (ppm): 2.32 (s, 3H, CH<sub>3</sub>),  
3.52(s, 2H, CH<sub>2</sub>), 6.88 (s, 1H, CH), 7.0(s,  
1H, CH) 7.12(d, *J*= 7.5 Hz, 2H, Ar-H), 7.35(d, *J*=8.0Hz,  
4H, Ar- H), 7.45 (s, *J*=8.0Hz 4H, CH), 8.03(s, 1H, NH).

**Elemental Analysis**

Calculated : C, 56.17; H, 4.38; N, 23.40

Found : C, 56.15; H, 4.37; N, 23.38

**4.1.9. Synthesis of 2-(morpholino)-N-(5-p-tolyl-1,3,4-thiadiazol-2-yl) acetamide (TZ 5).**

Yield : 1.88 g; 70.5 %

Melting Point : 204-206 °C

Rf Value : 0.74 (benzene: ethyl acetate (8:2))

Molecular Formula : C<sub>15</sub>H<sub>18</sub>N<sub>4</sub>O<sub>2</sub>S

Molecular Weight : 318 (M<sup>+</sup>)

IR (KBr) cm<sup>-1</sup> : 3465(NH), 3058 (Ar-CH), 3058(CH<sub>3</sub>), 1702(C=O),  
1620(C=N Str), 689 (C-S-C).

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ ppm: <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ (ppm): 2.34 (s,3H,CH<sub>3</sub>),2.37  
(s,2H,CH<sub>2</sub>), 3.25(s,2H,CH<sub>2</sub>),3.67(d,2H,CH<sub>2</sub>), 7.12(m,  
*J*=7.5Hz,4H,Ar-H),7.34(m,*J*=8.0Hz,8H,Ar-H),  
8.05(s,1H,NH).

Elemental Analysis

Calculated : C, 56.58; H, 5.70; N, 17.60.

Found : C, 56.56; H, 5.69; N, 17.58.



**4.1.10. Synthesis of N-(5-(4-bromophenyl)-1,3,4-thiadiazol-2-yl)-2  
(dimethylamino) acetamide (TZ 6).**

Yield	: 1.92 g; 80.1 %
Melting Point	: 157-159 °C
Rf Value	: 0.75 (benzene: ethyl acetate (8:2))
Molecular Formula	: C <sub>12</sub> H <sub>13</sub> BrN <sub>4</sub> OS
Molecular Weight	: 341(M <sup>+</sup> ), 343 (M+2)
IR (KBr) cm <sup>-1</sup>	: 3420(NH), 3050 (Ar-CH), 2816(CH <sub>3</sub> ), 1713(C=O), 1626(C=N Str), 689 (C-S-C), 654(C-Br).
<sup>1</sup> H NMR (CDCl <sub>3</sub> ) δ ppm	: <sup>1</sup> H NMR (CDCl <sub>3</sub> ) δ (ppm): 2.27 (d, 6H, CH <sub>3</sub> ), 3.24 (s, 2H, CH <sub>2</sub> ), 7.37 (d, <i>J</i> = 8.0Hz, 2H, Ar-H), 7.69 (d, 2H, Ar-H), 8.02 (s, 1H, NH).
Elemental Analysis	
Calculated	: C, 42.24; H, 3.84; N, 16.42.
Found	: C, 42.22; H, 3.84; N, 16.40.

**4.1.11. Synthesis of N-2-(Pyrolidino) (5-(4-bromophenyl)-1, 3, 4-thiadiazol-2-yl) acetamide (TZ 7).**

Yield	: 1.58 g; 62.8 %
Melting Point	: 130-134 °C
Rf Value	: 0.59 (benzene: ethyl acetate (8:2))
Molecular Formula	: C <sub>14</sub> H <sub>15</sub> BrN <sub>4</sub> OS
Molecular Weight	: 367(M <sup>+</sup> ), 369(M+2)
IR (KBr) cm <sup>-1</sup>	: 3442(NH), 3042(Ar-CH), 2946(CH <sub>2</sub> ), 1704(C=O), 1647 (C=Nstr), 672 (C-S-C), 654(C-Br).
<sup>1</sup> H NMR (CDCl <sub>3</sub> ) δ ppm	: <sup>1</sup> H NMR (CDCl <sub>3</sub> ) δ (ppm): 1.59 (d, 2H, CH <sub>2</sub> ), 2.25 (d, 1H, OH), 3.23 (s, 2H, CH <sub>2</sub> ), 7.36(d, <i>J</i> =8.0 Hz, 2H, Ar-H), 7.48(d, <i>J</i> =8.0 Hz, 2H, Ar-H), 8.01(s, 1H, NH).
Elemental Analysis	
Calculated	: C, 45.78; H, 4.12; N, 15.26.
Found	: C, 45.76; H, 4.11; N, 15.25.

**4.1.12. Synthesis of N-2-(Piperidino)(5-(4-bromophenyl)-1, 3, 4-thiadiazol-2-yl) acetamide (TZ 8).**

Yield	: 1.89 g; 76.5 %
Melting Point	: 212-214 °C
Rf Value	: 0.88 (benzene: ethyl acetate (8:2))
Molecular Formula	: C <sub>9</sub> H <sub>13</sub> BrN <sub>4</sub> OS
Molecular Weight	: 305 (M <sup>+</sup> ); 307(M+2)
IR (KBr) cm <sup>-1</sup>	: 3443 (NH),3057 (Ar-CH), 2989(CH <sub>3</sub> )1714(C=O),1629 (C=N Str), 689 (C-S- C),659(C-Br).
<sup>1</sup> H NMR (CDCl <sub>3</sub> ) δ ppm	: <sup>1</sup> H NMR (CDCl <sub>3</sub> ) δ (ppm): 1.52( d, 2H,CH <sub>2</sub> ), 2.24 (s, 2H, CH <sub>2</sub> ), 6.21 (d,8H, CH <sub>2</sub> ), 7.37 (d, <i>J</i> = 8.0 Hz, 2H, Ar-H), 7.45 (d, <i>J</i> = 8.0 Hz, 2H, Ar-H), 8.02(s,1H,NH).
Elemental Analysis	
Calculated	: C, 35.42; H, 4.29; N, 18.36.
Found	: C, 35.40; H, 4.28; N, 18.34.

**4.1.13. Synthesis of N-2-(Piperidino)(5-(4-bromophenyl)-1, 3, 4-thiadiazol-2-yl) acetamide (TZ 9).**

Yield	: 1.46 g; 80.7 %
Melting Point	: 205-207 °C
Rf Value	: 0.79 (benzene: ethyl acetate (8:2))
Molecular Formula	: C <sub>7</sub> H <sub>6</sub> BrN <sub>5</sub> OS
Molecular Weight	: 288 (M <sup>+</sup> ); 290 (M+2)
IR (KBr) cm <sup>-1</sup>	: 3478 (NH), 3050 (Ar-CH), 2929(CH <sub>3</sub> ) 1710(C=O), 1626 (C=N Str), 689 (C-S-C), 653(C-Br).
<sup>1</sup> H NMR (CDCl <sub>3</sub> ) δ ppm	: <sup>1</sup> H NMR (CDCl <sub>3</sub> ) δ (ppm): 3.44 (s, 2H, CH <sub>2</sub> ), 6.21 (d, 2H, CH <sub>2</sub> ), 7.01 (s, 2H, CH), 7.36(d, <i>J</i> = 8.0 Hz, 2H, Ar- H), 7.47 (d, <i>J</i> = 7.0 Hz, 2H, Ar-H), 8.0(s, 1H, NH).
Elemental Analysis	
Calculated	: C, 29.18; H, 2.10; N, 24.31.
Found	: C, 29.15; H, 2.10; N, 24.29.

**4.1.14. Synthesis of N-2-(Morpholino) (5-(4-bromophenyl)-1, 3, 4-thiadiazol-2-yl) acetamide (TZ 10).**

Yield	: 1.65 g; 73.8 %
Melting Point	: 226-228 °C
Rf Value	: 0.83 (benzene: ethyl acetate (8:2))
Molecular Formula	: C <sub>8</sub> H <sub>11</sub> BrN <sub>4</sub> O <sub>2</sub> S
Molecular Weight	: 307 (M <sup>+</sup> ); 309 (M+2)
IR (KBr) cm <sup>-1</sup>	: 3474 (NH), 3058 (Ar-CH), 2876(CH <sub>3</sub> ), 1712(C=O), 1620 (C=N Str), 689 (C-S-C), 655(C-Br).
<sup>1</sup> H NMR (CDCl <sub>3</sub> ) δ ppm	: <sup>1</sup> H NMR (CDCl <sub>3</sub> ) δ (ppm): 2.90 (d, 4H, CH <sub>2</sub> ), 3.42 (s, 2H, CH <sub>2</sub> ), 3.65 (s, 4H, CH <sub>2</sub> ), 7.37-(d, <i>J</i> = 8.0 Hz, 2H, Ar-H), 7.49 (d, <i>J</i> = 7.0 Hz, 2H, Ar-H), 8.02(s, 1H, NH).
Elemental Analysis	
Calculated	: C, 31.28; H, 3.61; N, 18.24.
Found	: C, 31.26; H, 3.61; N, 18.22.

## **4.2. CHROMATOGRAPHY STUDIES OF SYNTHESIZED COMPOUNDS**

### **4.2.1 THIN LAYER CHROMATOGRAPHY**

Thin Layer Chromatography or TLC is a solid-liquid form of chromatography here the stationary phase is a polar absorbent and the mobile phase can be a single solvent or Combination of solvents. TLC is inexpensive technique and quick that can be used for determine the number of components in a mixture, verify a substance's identity, monitor the process of a reaction, determine appropriate condition for column chromatography, analyze the fractions obtained from column chromatography.

#### **4.2.1.1 MATERIALS AND METHODS**

##### **1. Preparation of plates**

Silica gel G was mixed in a glass mortar to smooth consistency with the requisite amount of water and slurry was quickly transferred to the spreader. The mixtures have been spread over the plates in thickness of 0.2 mm and allow setting into a suitable holder and after 30 minutes, plates were dried at 120°C, for further activation of the absorbent.

##### **2. Sample application**

About 2 mm of absorbent from the edge of plate was removed to give sharply defined edges. 2-5  $\mu$ l volumes of synthesized compounds were spotted with the help of capillary tubes, just above 1 cm of the bottom of coated plates.

##### **3. Development chamber**

The chromatographic chamber was lined with filter paper dipping into mobile phase so as to maintain the atmospheric saturation with solvent vapors in the chamber.

#### **4. Solvent system**

The choice of best developing solvent is one of the most important decisions in practical TLC by review of literature survey on by knowing nature of compounds, this solvent system used is benzene: ethyl acetate (8:2).

#### **5. Detection of components**

The spots were visualized under Iodine chamber.

#### **4.2.2 COLUMN CHROMATOGRAPHY**

Purification of synthesized derivatives was done by column chromatography.

##### **Materials**

1. Glass column of size 45 cm x 3cm.
2. Silica gel for column chromatography 60-120 mesh size.
3. Eluting solvent system benzene: ethyl acetate (8:2).

##### **Preparation of column**

The silica gel 60-120 mesh size was made into slurry with the above solvent system. The bottom of the column was plugged with little glass wool. Then the slurry was poured into the column, which is filled with solvent after two third of the column areas were filled with slurry. It was set aside for 30 minutes and eluting solvent was passed through column for several times ensure good packing of the column. After the adsorbents are settled, a filter paper was kept to prevent disturbance of the top layer of the adsorbent as fresh mobile phase to be added to column for the process of elution. The fractions were collected for every 5 ml and analyzed for the presence of different of similar compound by running TLC and then allow evaporating to get the residue.

### **4.3. PHARMACOLOGICAL SCREENING**

#### **4.3.1. *IN-VITRO* ANTI-CANCER ACTIVITY**

Tissue culture has been used to screen many anti cancer drugs since there is clear correlation between the in vitro and in vivo activities of potential chemotherapeutic agents. There is scientific justification for cytotoxicity testing in tissue, since animal models are in many ways inadequate for predicting the effects of chemicals on humans since there are many metabolic differences between species.

Cytotoxicity studies involve the analysis of morphological damage or inhibition of zone of outgrowth induced by the chemicals tested.

#### **ASSAY FOR PROLIFERATION STUDIES**

##### **IN VITRO ANTI CANCER ACTIVITY**

The human cervical cancer cell line (HeLa) was obtained from national center for cell science (NCCS), pune. The HeLa cells were grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS) and maintained at 37°C, 5% CO<sub>2</sub>, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

Toxicity of test compound in cells was determined by MTT assay based on mitochondrial reduction of yellow MTT tetrazolium dye to a highly colored blue formazan product<sup>40</sup>.



## **Assay for Proliferation Studies - MTT Assay**

### **Principle**

MTT [(3-(4,5-dimethyl thiazol-2yl)-2,5diphenyl tetrazolium bromide)] measures the metabolic activity of the viable cells. The assay can be performed entirely in a microtiterplate (MTP). It is suitable for measuring cell proliferation, Cell viability or Cytotoxicity. The reaction between MTT and mitochondrial dehydrogenase produces water-insoluble formazan salt. This method involves culturing the cells in a 96 well microtiterplate and then incubating with MTT solution for approximately 2 hours. During incubation period, viable cells convert MTT to a water insoluble formazan dye. The formazan dye in the MTP is solubilized and quantified with an ELISA plate reader. The absorbance directly correlates with the cell number. This is applicable for adherent cells cultured in MTP.

### **Materials for MTT assay**

- The human cervical cancer cell line (**HeLa**)
- Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS)
- Phosphate buffered saline (PBS)
- Dimethyl sulphoxide (DMSO)
- MTT [(3-(4,5-dimethylthiazol-2yl)-2,5 diphenyltetrazoliumbromide)]
- CO<sub>2</sub> incubator (WTC Binder, Germany)

- Laminar air flow cabin (Klenzaids, Chennai, India )
- Refrigerated centrifuge ( Biofuge fresco, Heraeus, Germany )
- ELISA-reader ( For MTP ) Anthos 2010, Germany )
- Deep freezer (Polar Angelantoni Industries, Italy )
- Ultrasonic bath ( Transonic [ 460/H ], by Elma, Germany ]
- Vacuum pump ( Zenith [model: PDF-2-2.5], Mumbai, India )
- Pipettes (Eppendorf, Hamburg, Germany )
- Culture plates
- Centrifuge tubes
- Aerosol resistant tips
- Flat-bottomed 96-MTP
- Tissue culture grade

### **Cell treatment procedure**

Cell treatment procedure The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium with 5% FBS to give final density of  $1 \times 10^5$  cells/ml. one hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of

10,000 cells/well and incubated to allow for cell attachment at 37°C, 5% CO<sub>2</sub>, 95% air and 100% relative humidity.

After 24 h the cells were treated with serial concentrations of the extracts and fractions. They were initially dissolved in neat dimethylsulfoxide (DMSO) and further diluted in serum free medium to produce five concentrations. One hundred microlitres per well of each concentration was added to plates to obtain final concentrations of 100, 10, 1.0 and 0.1 µM. The final volume in each well was 200 µl and the plates were incubated at 37°C, 5% CO<sub>2</sub>, 95% air and 100% relative humidity for 48h. The medium containing without samples were served as control. Triplicate was maintained for all concentrations.

## **Procedure**

### ***In-vitro* anticancer screening**

The human cervical cancer cell line (**HeLa**) was obtained from National Centre for Cell Science (NCCS), Pune. The cells were grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS).

For screening experiment, the cells were seeded into 96-well plates in 100µl of medium containing 5 % FBS, at plating density of 10,000 cells/well and incubated at 37 °C, 5 % CO<sub>2</sub>, 95 % air and 100 % relative humidity for 24 hours prior to addition of samples. The samples were solubilized in Dimethyl sulfoxide and diluted in serum free medium. After 24 hours, 100 µl of the medium containing the samples at various concentration ( eg; 0.063, 0.125, 0.25, 0.5, 1.0 mM etc... ) was added and incubated at 37°C, 5% CO<sub>2</sub>, 95% air and 100% relative humidity for 48 hours.

Triplicate was maintained and the medium containing without samples were served as control<sup>41</sup>.

After 48 hours, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37 °C for 4 hours. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula.

$$\% \text{ cell Inhibition} = 100 - \{(\text{sample}) / \text{Abs (control)}\} \times 100$$

Nonlinear regression graph was plotted between % Cell inhibition and Log10 concentration and IC50 was determined using GraphPad Prism software.

### **Statistical Analysis**

All values are expressed as mean ± SEM. Data were analyzed by non-parametric ANOVA followed by Dunnett's multiple comparison tests, and other data was evaluated using Graph Pad PRISM software. A *p*-value < 0.05 was considered significantly different.

## CHAPTER – 5

### RESULTS AND DISCUSSION

#### 5.1. Chemical work:

The results of the present work are discussed under the following heads.

##### Scheme:

Synthesis of substituted N-(5-aryl-1, 3, 4-thiadiazole-2-yl)-2-alkyl / aryl substituted acetamides.

**5.1.1.** Synthesis of substituted N-(5-aryl-1, 3, 4-thiadiazole-2-yl)-2-alkyl / aryl substituted acetamides. Synthetic route depicted in scheme outline the chemistry part of the present work. The N-(5-aryl-1, 3, 4-thiadiazole-2-yl)-2-alkyl / aryl substituted acetamides (**TZ1-10**) were obtained by the condensation of N-(5-aryl-1, 3, 4-thiadiazole-2-yl)-2-chloroacetamide with alkyl and aryl substituted derivatives in presence of ethanol. The formation of the thiadiazole was confirmed by the presence of characteristic peaks in the IR spectra. It showed characteristic peaks at around  $3400\text{ cm}^{-1}$  for  $\text{NH}_2$  stretching and peak around  $2900\text{ cm}^{-1}$  due to the presence of  $\text{N}=\text{CH}$  stretching. The NMR spectrum of the compounds TZ1-10 showed the characteristic peak around  $\delta\ 2.70\text{ ppm}$  for  $\text{CH}_3$  group,  $\delta\ 3.00\text{ ppm}$  for  $\text{CH}_2$  and  $\delta\ 5.70\text{ ppm}$  for  $\text{NCH}$  and also shows multiplet in the range of  $\delta\ 6.80\text{--}7.80\text{ ppm}$  owing to aromatic protons. The appearance of peak due to chlorine, bromine and fluorine in IR spectra around  $700\text{--}800\text{ cm}^{-1}$  and formation  $\text{M}+2$  peak in the mass spectra. Data from the elemental analyses and molecular ion recorded in the mass spectra further confirmed the assigned structure.

#### 5.2. Pharmacological Investigation

The anticancer screening of title compounds (TZ1-10) were evaluated against human cervical cancer cell line (HeLa) by MTT assay method. In this assay the effective ranges of anticancer activity for compounds TZ1-10 were in the concentration of 0.1, 1.0, 10, 100  $\mu\text{M}$  respectively in the human cervical cancer cell line (HeLa). Triplicate was maintained and the medium containing without samples were served as control.

TZ1 (dimethylamino) produced IC<sub>50</sub> value 45.70  $\mu$ M in case of the human cervical cancer cell line (HeLa). Relatively less value of IC<sub>50</sub> indicates the sample has more anticancer activity. The compound TZ1 (dimethylamino) had shown the percentage of cell inhibition was 74.85 against the human cervical cancer cell line (HeLa) in the highest concentration, which have dimethylamino group in the thiadiazole nucleus.

The result indicates that TZ1 (dimethylamino group) showed a high significant anticancer activity against the human cervical cancer cell line (HeLa), when compared to that control.

TZ2 (pyrrolidine) produced IC<sub>50</sub> value 66.23  $\mu$ M in case of the human cervical cancer cell line (HeLa). Relatively less value of IC<sub>50</sub> indicates the sample has more anticancer activity. The compound TZ2 (pyrrolidine) had shown the percentage of cell inhibition was 52.89 against the human cervical cancer cell line (HeLa) in the highest concentration, which have pyrrolidine group in the thiadiazole nucleus. The results indicate that TZ2 (pyrrolidine group) showed a moderate anticancer activity against the human cervical cancer cell line (HeLa), when compared to that of control.

TZ3 (piperidine) produced IC<sub>50</sub> value >100 $\mu$ M in case of the human cervical cancer cell line (HeLa). Relatively less value of IC<sub>50</sub> indicates the sample has more anticancer activity. The compound TZ2 (piperidine) had shown the percentage of cell inhibition was 51.30 against the human cervical cancer cell line (HeLa), which have piperidine group in the thiadiazole nucleus.

The results indicate that TZ3 (piperidine group) showed a less anticancer activity against the human cervical cancer cell line (HeLa), when compared to that of control.

TZ4 (imidazole) produced IC<sub>50</sub> value 68.25  $\mu$ M in case of the human cervical cancer cell line (HeLa). Relatively less value of IC<sub>50</sub> indicates the sample has more anticancer activity. The compound TZ4 (imidazole) had shown the percentage of cell inhibition was 62.85 against the human cervical cancer cell line (HeLa) in the highest concentration, which have imidazole group in the thiadiazole nucleus.

The results indicate that TZ4 (imidazole group) showed a moderate significant anticancer activity against the human cervical cancer cell line (HeLa), when compared to that of control.

TZ5 (morpholine) produced IC<sub>50</sub> value 47.79  $\mu$ M in case of the human cervical cancer cell line (HeLa). Relatively less value of IC<sub>50</sub> indicates the sample has more anticancer activity. The compound S5 (Nitro) had shown the percentage of cell inhibition was 70.89 against the human cervical cancer cell line (HeLa) in the highest concentration, which have morpholine group in the thiadiazole nucleus.

The results indicate that TZ5 (morpholine group) showed a good significant anticancer activity against the human cervical cancer cell line (HeLa), when compared to that of control.

TZ6 (dimethyl) produced IC<sub>50</sub> value 48.60  $\mu$ M in case of the human cervical cancer cell line (HeLa). Relatively less value of IC<sub>50</sub> indicates the sample has more anticancer activity. The compound TZ6 (dimethyl) had shown the percentage of cell inhibition was 73.85 against the human cervical cancer cell line (HeLa) in the highest concentration, which have dimethyl group in the thiadiazole nucleus.

The results indicate that TZ6 (dimethylamino group) showed a good significant anticancer activity against the human cervical cancer cell line (HeLa), when compared to that of control.

TZ7 (pyrrolidine) produced IC<sub>50</sub> value 75.26  $\mu$ M in case of the human cervical cancer cell line (HeLa). Relatively less value of IC<sub>50</sub> indicates the sample has more anticancer activity. The compound TZ7 (dimethyl) had shown the percentage of cell inhibition was 55.05 against the human cervical cancer cell line (HeLa) in the highest concentration, which have dimethyl group in the thiadiazole nucleus.

The results indicate that TZ7 (pyrrolidine group) showed a less significant anticancer activity against the human cervical cancer cell line (HeLa), when compared to that of control.

TZ8 (piperidine) produced IC<sub>50</sub> value 92.36  $\mu$ M in case of the human cervical cancer cell line (HeLa). Relatively less value of IC<sub>50</sub> indicates the sample has more anticancer activity. The compound TZ8 (dimethyl) had shown the percentage of cell inhibition was 60.82 against the human cervical cancer cell line (HeLa) in the highest concentration, which have dimethyl group in the thiadiazole nucleus.

The results indicate that TZ8 (piperidine group) showed a less significant anticancer activity against the human cervical cancer cell line (HeLa), when compared to that of control.

TZ9 (imidazole) produced IC<sub>50</sub> value 70.48  $\mu$ M in case of the human cervical cancer cell line (HeLa). Relatively less value of IC<sub>50</sub> indicates the sample has more anticancer activity. The compound TZ9 (dimethyl) had shown the percentage of cell inhibition was 60.01 against the human cervical cancer cell line (HeLa) in the highest concentration, which have imidazole group in the thiadiazole nucleus.

The results indicate that TZ9 (imidazole group) showed a less significant anticancer activity against the human cervical cancer cell line (HeLa), when compared to that of control.

TZ10 (morpholine) produced IC<sub>50</sub> value 49.26  $\mu$ M in case of the human cervical cancer cell line (HeLa). Relatively less value of IC<sub>50</sub> indicates the sample has more anticancer activity. The compound TZ10 (morpholine) had shown the percentage of cell inhibition was 70.12 against the human cervical cancer cell line (HeLa) in the highest concentration, which have imidazole group in the thiadiazole nucleus.

The results indicate that TZ10 (morpholine group) showed a less significant anticancer activity against the human cervical cancer cell line (HeLa), when compared to that of control.

The best mean IC<sub>50</sub> values were achieved with compound (TZ1, TZ5, TZ6 and TZ10) with slight difference among them.



Title compounds (TZ1-10) were found to exhibit mild to moderate anticancer activities in cell lines and the results were summarized below:

- Compound TZ1 (dimethylamino) shows the high significant activity against the HeLa (IC<sub>50</sub> – 47.50) cancer cell lines.
- Compound TZ2 (pyrrolidine) shows the significant activity against the HeLa (IC<sub>50</sub> – 66.23) cancer cell lines.
- Compound TZ3 (piperidine) shows the no action against the HeLa (IC<sub>50</sub> > 100) cancer cell lines.
- Compound TZ4 (imidazole) shows the moderate significant against the HeLa (IC<sub>50</sub> – 67.668.25) cancer cell lines.
- Compound TZ5 (morpholine) shows the high significant activity against the HeLa (IC<sub>50</sub> – 47.79) cancer cell lines.
- Compound TZ6 (dimethylamino) shows the moderate significant against the HeLa (IC<sub>50</sub> – 47.79) cancer cell lines.
- Compound TZ7 (pyrrolidine) shows the less significant against the HeLa (IC<sub>50</sub> – 75.26) cancer cell lines.
- Compound TZ8 (piperidine) shows the less significant against the HeLa (IC<sub>50</sub> – 92.36) cancer cell lines.
- Compound TZ9 (imidazole) shows the less significant against the HeLa (IC<sub>50</sub> – 70.48) cancer cell lines.
- Compound TZ10 (morpholine) shows the significant activity against the HeLa (IC<sub>50</sub> – 49.26) cancer cell lines.

Among the test compounds, compound 2-(dimethylamino)-N-(5-p-tolyl-1,3,4-thiadiazol-2-yl)acetamide(TZ1) was found to be the most active agent which showed 74.85 percentage of cell inhibition against the human cervical cancer cell line (HeLa) in the highest concentration, which have dimethylamino group in the thiadiazole nucleus.

*In vitro* cytotoxicity studies on Human cervical cancer cell line (HeLa)

## PERCENTAGE OF CELL INHIBITION:

Table No. 2

Compounds	Concentration	% cell Inhibition	Compounds	Concentration	% cell Inhibition
<b>TZ1</b>	0.1 $\mu$ M	1.3342	<b>TZ6</b>	0.1 $\mu$ M	1.2342
	1 $\mu$ M	12.6079		1 $\mu$ M	12.3079
	10 $\mu$ M	33.8761		10 $\mu$ M	32.9861
	100 $\mu$ M	<b>74.8578</b>		100 $\mu$ M	<b>73.8578</b>
<b>TZ2</b>	0.1 $\mu$ M	1.0424	<b>TZ7</b>	0.1 $\mu$ M	3.1895
	1 $\mu$ M	10.4447		1 $\mu$ M	14.2646
	10 $\mu$ M	22.9848		10 $\mu$ M	23.6772
	100 $\mu$ M	52.8995		100 $\mu$ M	<b>55.0587</b>
<b>TZ3</b>	0.1 $\mu$ M	3.4112	<b>TZ8</b>	0.1 $\mu$ M	1.4315
	1 $\mu$ M	13.7176		1 $\mu$ M	19.6317
	10 $\mu$ M	33.8976		10 $\mu$ M	35.4864
	100 $\mu$ M	<b>51.3006</b>		100 $\mu$ M	<b>60.8275</b>
<b>TZ4</b>	0.1 $\mu$ M	1.5342	<b>TZ9</b>	0.1 $\mu$ M	1.4846
	1 $\mu$ M	12.9079		1 $\mu$ M	11.3278
	10 $\mu$ M	30.9861		10 $\mu$ M	30.3398
	100 $\mu$ M	<b>62.8578</b>		100 $\mu$ M	<b>60.0175</b>
<b>TZ5</b>	0.1 $\mu$ M	1.4424	<b>TZ10</b>	0.1 $\mu$ M	1.4579
	1 $\mu$ M	11.4447		1 $\mu$ M	11.0145
	10 $\mu$ M	28.9848		10 $\mu$ M	28.2358
	100 $\mu$ M	<b>70.8995</b>		100 $\mu$ M	<b>70.1268</b>

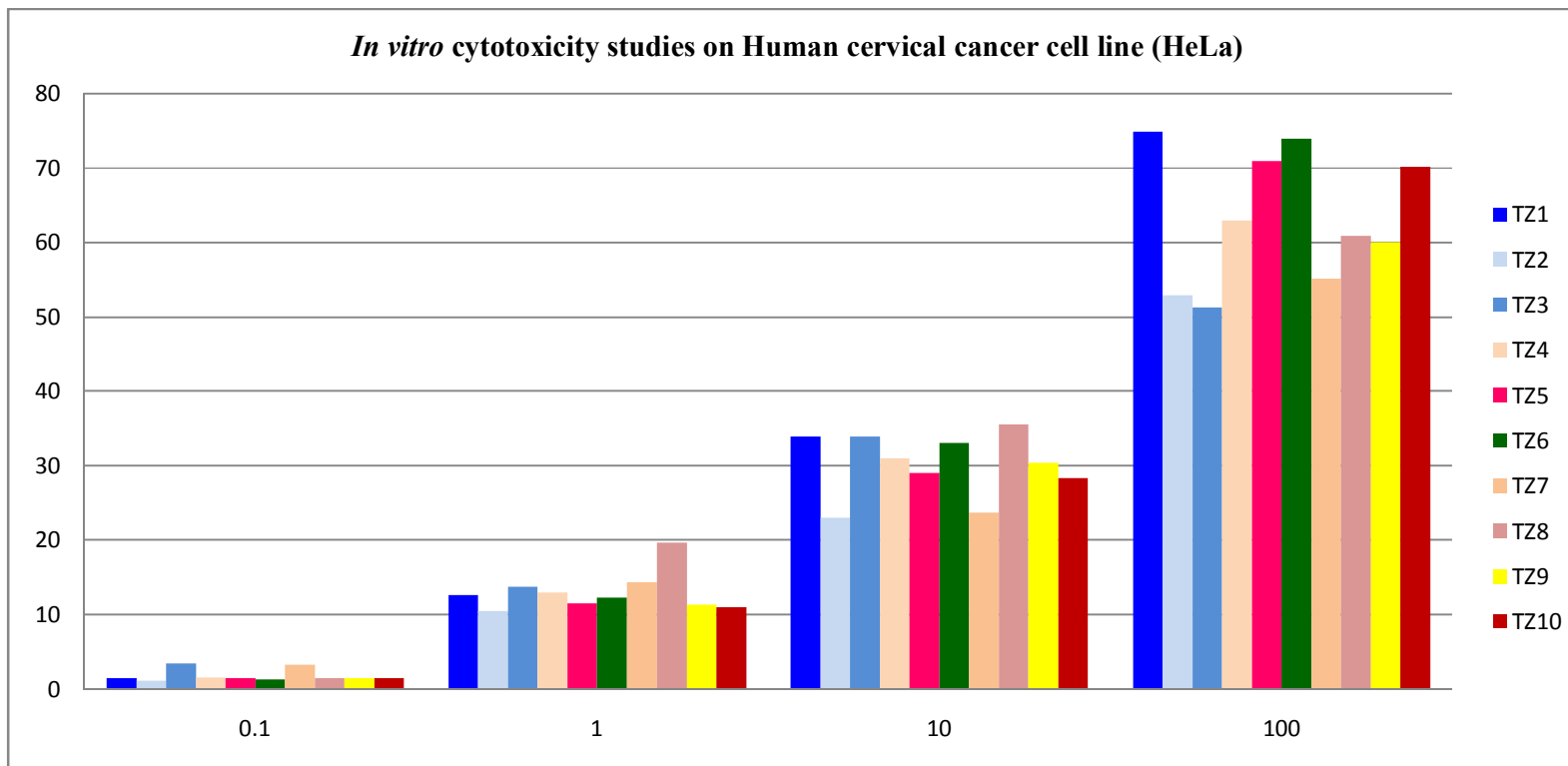
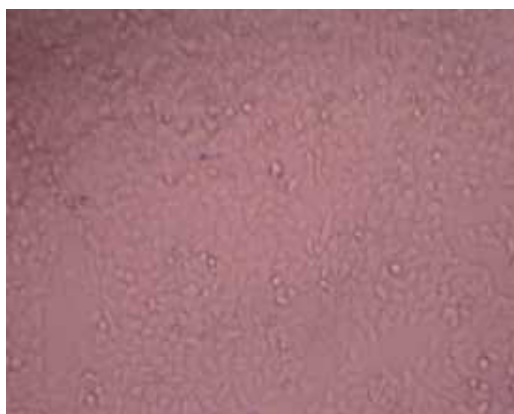


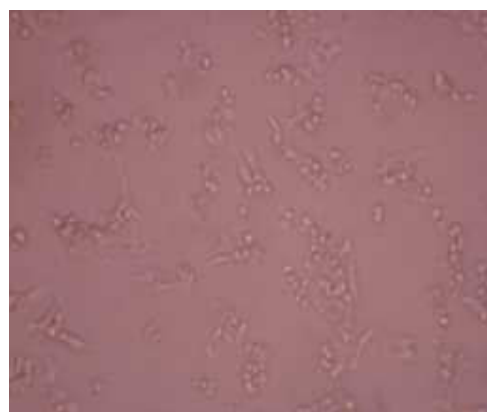
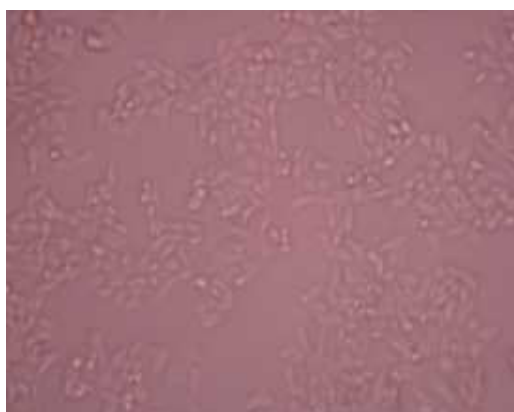
Fig.No.5. Percentage Cell Inhibition on Human Cervical Cancer Cell Line (HeLa)

**TZ1**



0.1  $\mu$ M

1  $\mu$ M



10  $\mu$ M

100  $\mu$ M



Normal

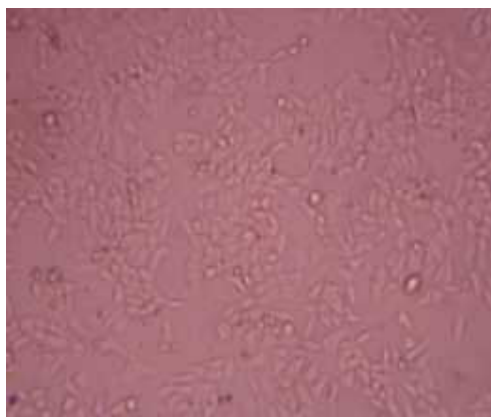
**TZ 2**



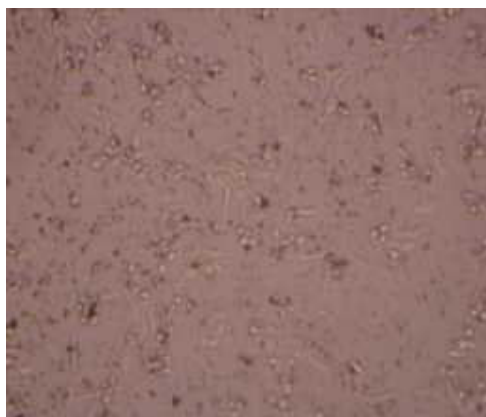
0.1  $\mu$ M



1  $\mu$ M



10  $\mu$ M

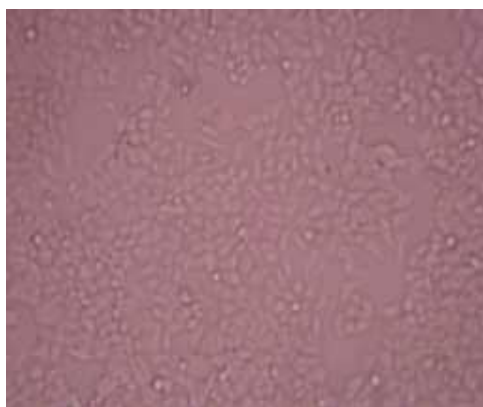


100  $\mu$ M



Normal

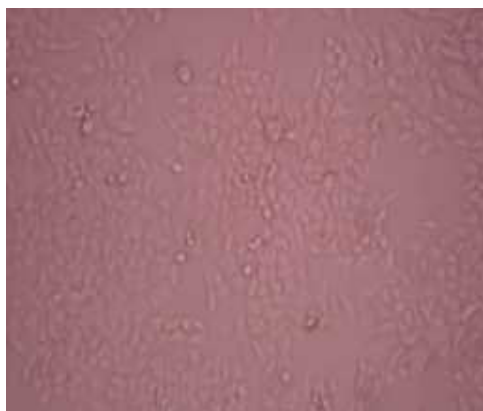
**TZ 3**



01.  $\mu\text{M}$



1  $\mu\text{M}$



10  $\mu\text{M}$



100  $\mu\text{M}$



Normal

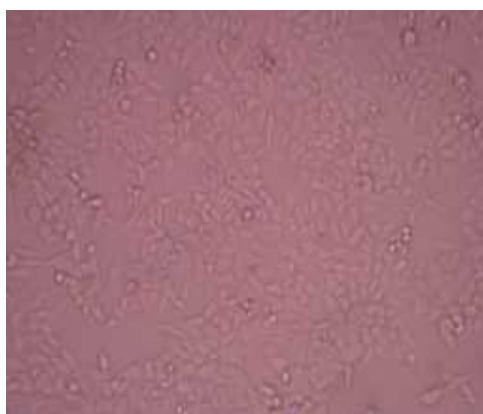
**TZ 4**



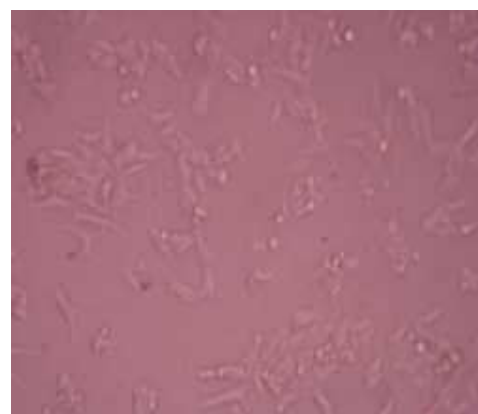
0.1  $\mu$ M



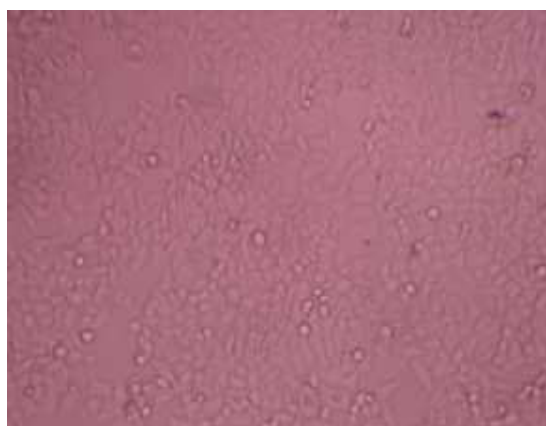
1  $\mu$ M



10  $\mu$ M

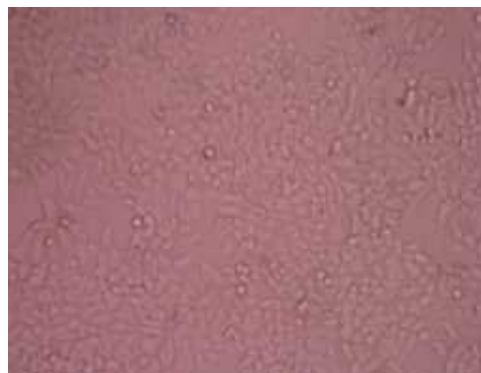


100  $\mu$ M

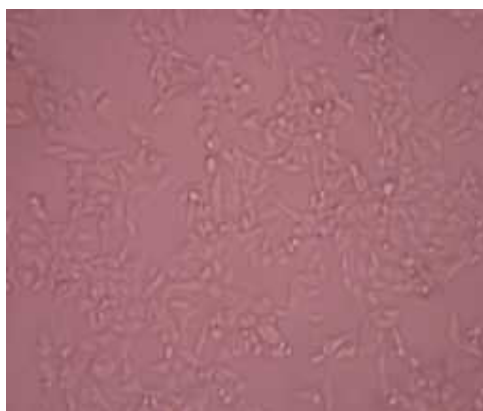


Normal

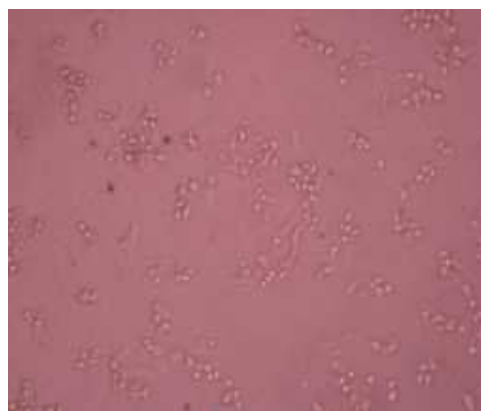
**TZ 5**



**0.1  $\mu$ M**



**1  $\mu$ M**



**10  $\mu$ M**

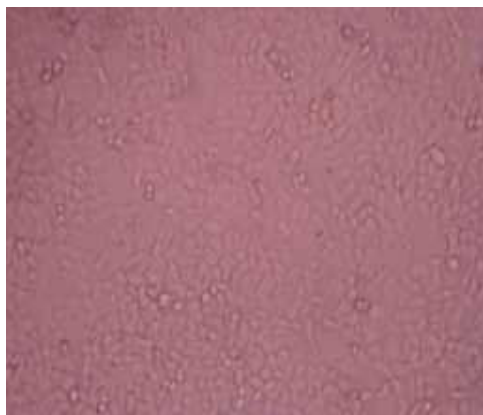
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**Normal**



**TZ 6**



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1  $\mu$ M



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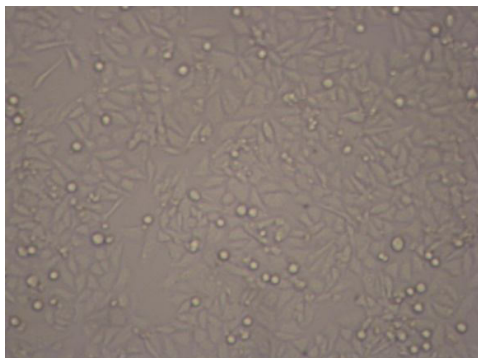


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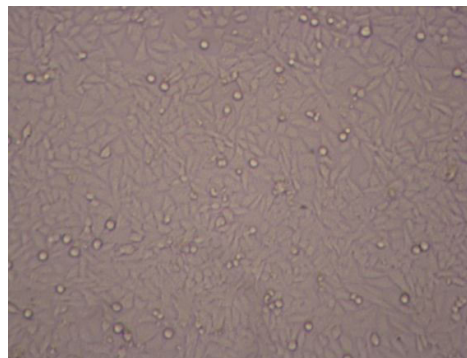


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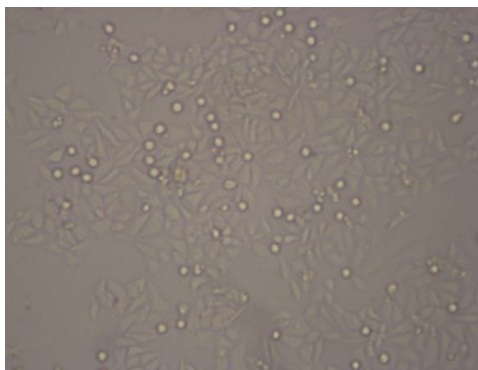
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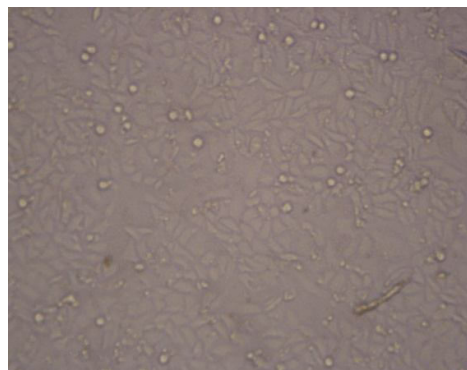
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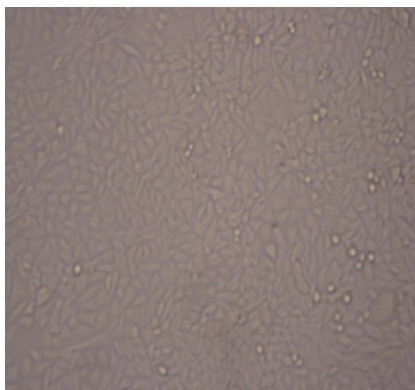
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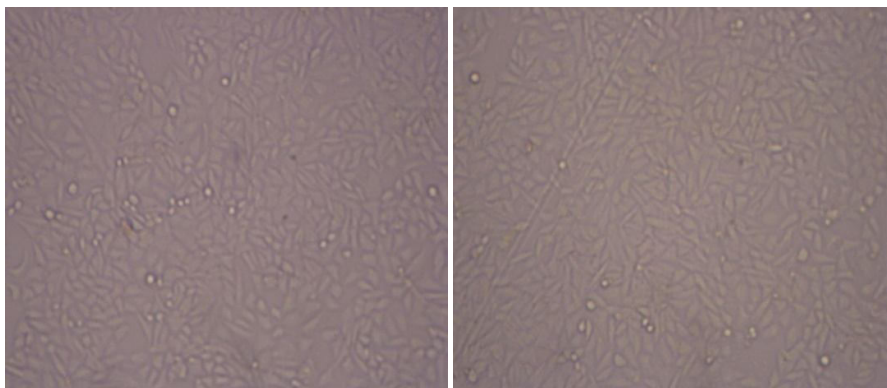


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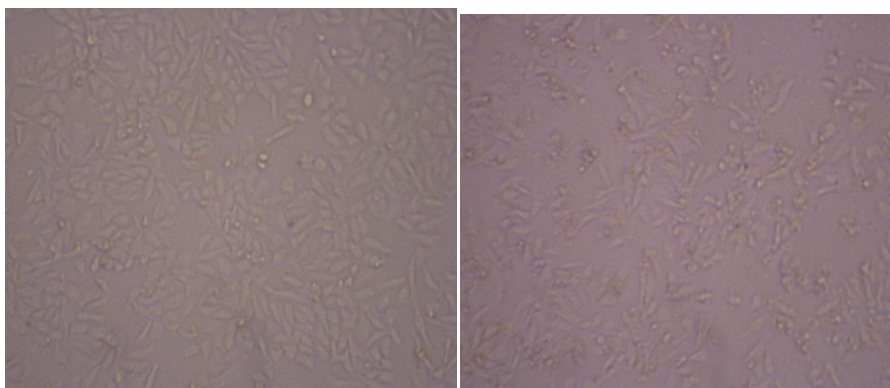
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**TZ 8**



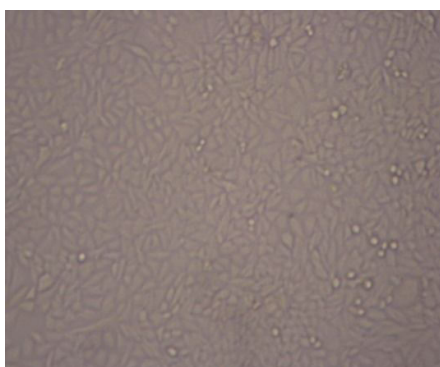
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1  $\mu$ M



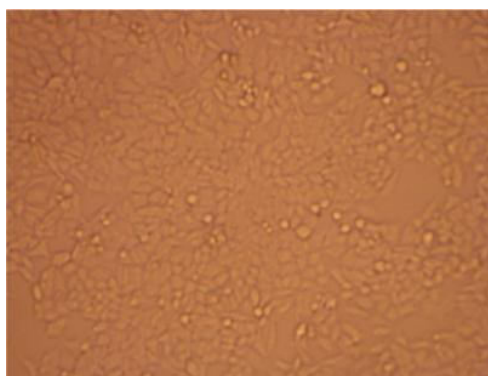
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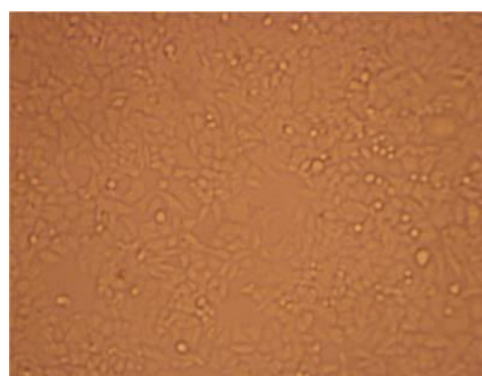


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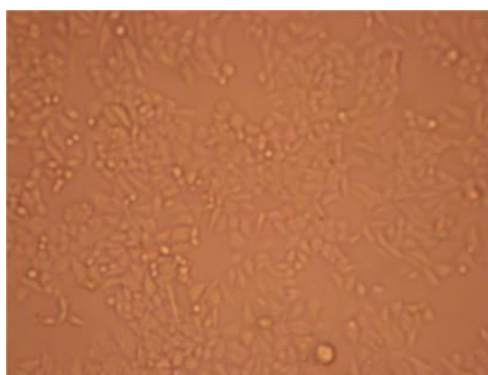
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0.1 $\mu$ M



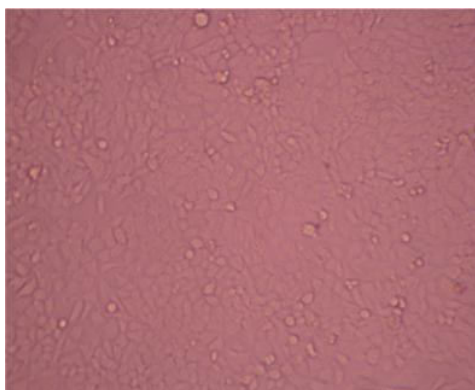
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10  $\mu$ M

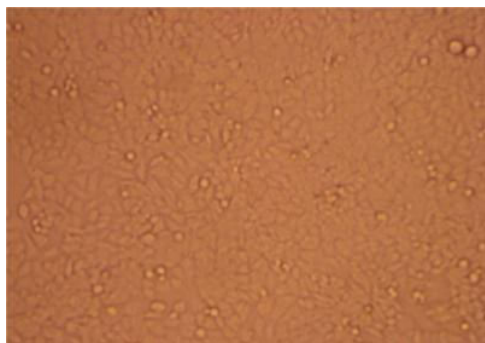


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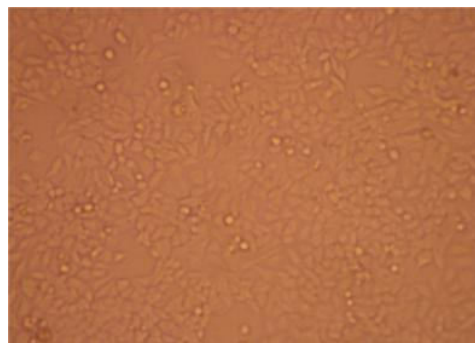


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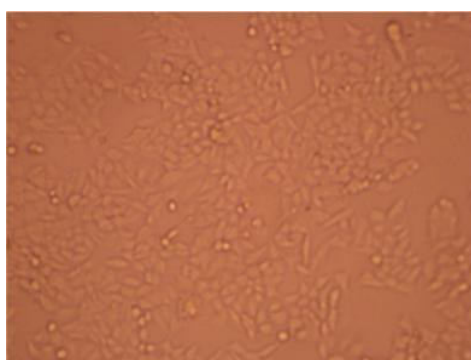
**TZ 10**



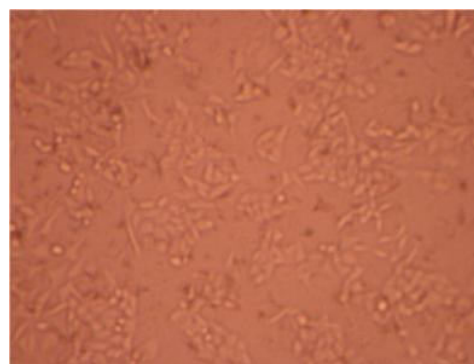
0.1  $\mu$ M



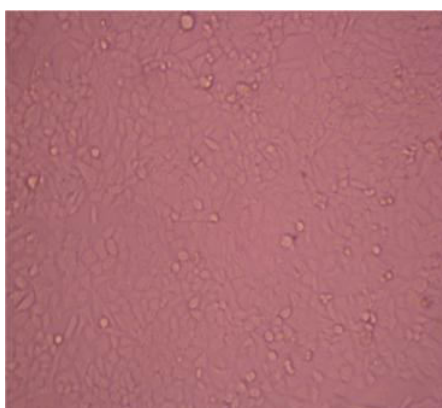
1  $\mu$ M



10  $\mu$ M



100  $\mu$ M



Normal

Table No.3: IC<sub>50</sub> Values of Synthesized Compounds (TZ1 – TZ10)

COMPOUND CODE	IC <sub>50</sub> (MICRO MOLAR)
TZ1	45.70 µM
TZ2	66.23 µM
TZ3	>100 µM
TZ4	68.25 µM
TZ5	47.79 µM
TZ6	48.60 µM
TZ7	75.26 µM
TZ8	92.36 µM
TZ9	70.48 µM
TZ10	49.26 µM

Table No.4. TZ1

Concentration ( $\mu\text{M}$ )	% Growth inhibition	IC <sub>50</sub>	R <sup>2</sup>
0.1 $\mu\text{M}$	1.3342	45.70	0.9995
1 $\mu\text{M}$	12.6079		
10 $\mu\text{M}$	33.8761		
100 $\mu\text{M}$	74.8578		

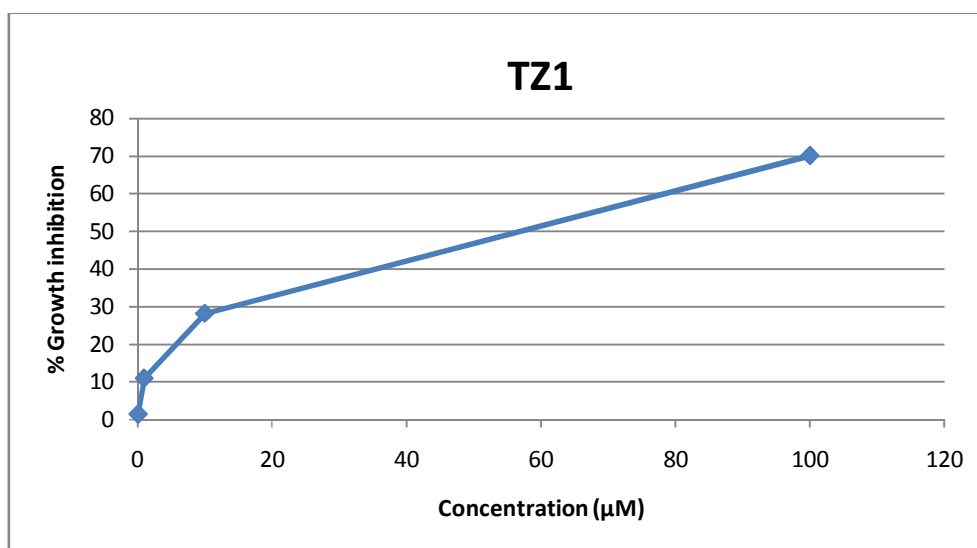


Table No. 5. TZ2

Concentration ( $\mu\text{M}$ )	% Growth inhibition	IC <sub>50</sub>	R <sup>2</sup>
0.1 $\mu\text{M}$	1.0424	66.23	0.9996
1 $\mu\text{M}$	10.447		
10 $\mu\text{M}$	22.9848		
100 $\mu\text{M}$	52.9885		

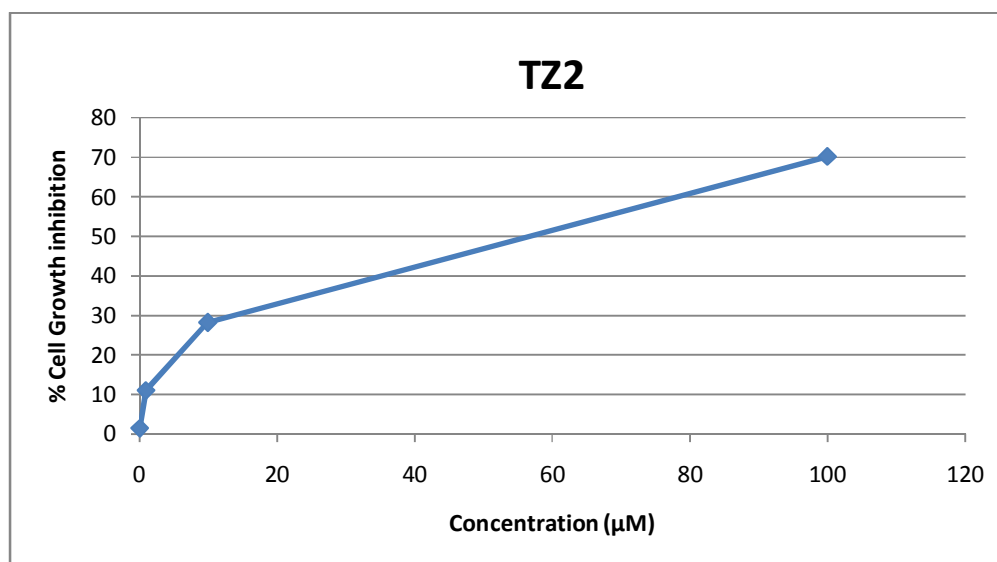




Table No. 6. TZ3

Concentration ( $\mu\text{M}$ )	% Growth inhibition	IC <sub>50</sub>	R <sup>2</sup>
0.1 $\mu\text{M}$	3.4112	>100	0.9907
1 $\mu\text{M}$	13.7176		
10 $\mu\text{M}$	33.8976		
100 $\mu\text{M}$	51.3006		

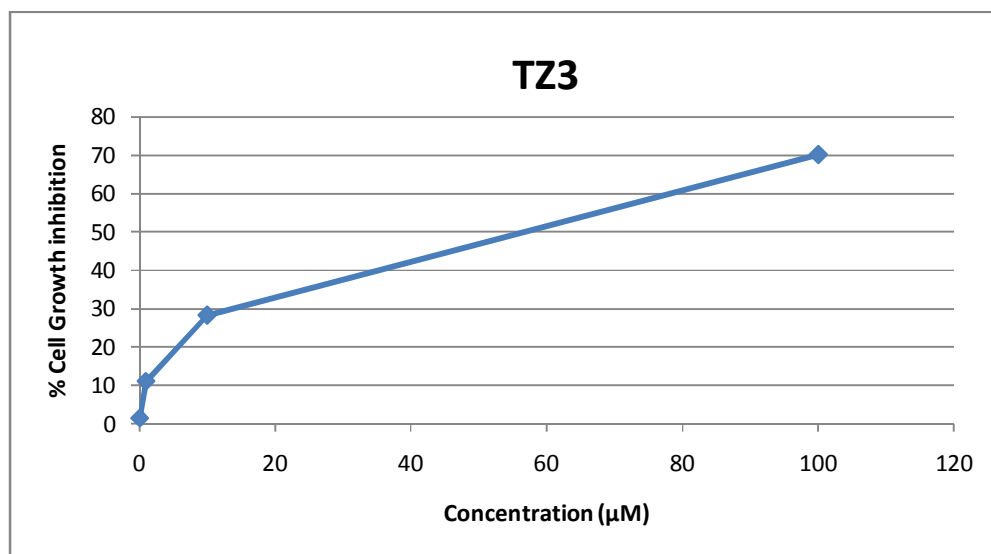


Table No. 7. TZ4

Concentration ( $\mu\text{M}$ )	% Growth inhibition	IC <sub>50</sub>	R <sup>2</sup>
0.1 $\mu\text{M}$	1.5342	68.25	0.9367
1 $\mu\text{M}$	12.9079		
10 $\mu\text{M}$	30.9861		
100 $\mu\text{M}$	62.8578		

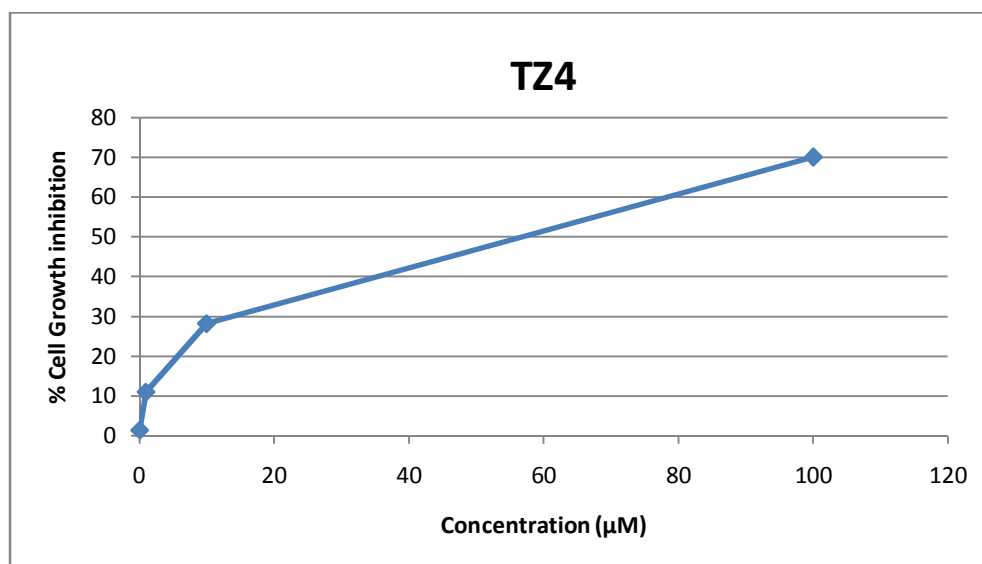


Table No. 8. TZ5

Concentration ( $\mu\text{M}$ )	% Growth inhibition	IC <sub>50</sub>	R <sup>2</sup>
0.1 $\mu\text{M}$	1.4424	47.79	0.9990
1 $\mu\text{M}$	11.4447		
10 $\mu\text{M}$	28.9848		
100 $\mu\text{M}$	70.8995		

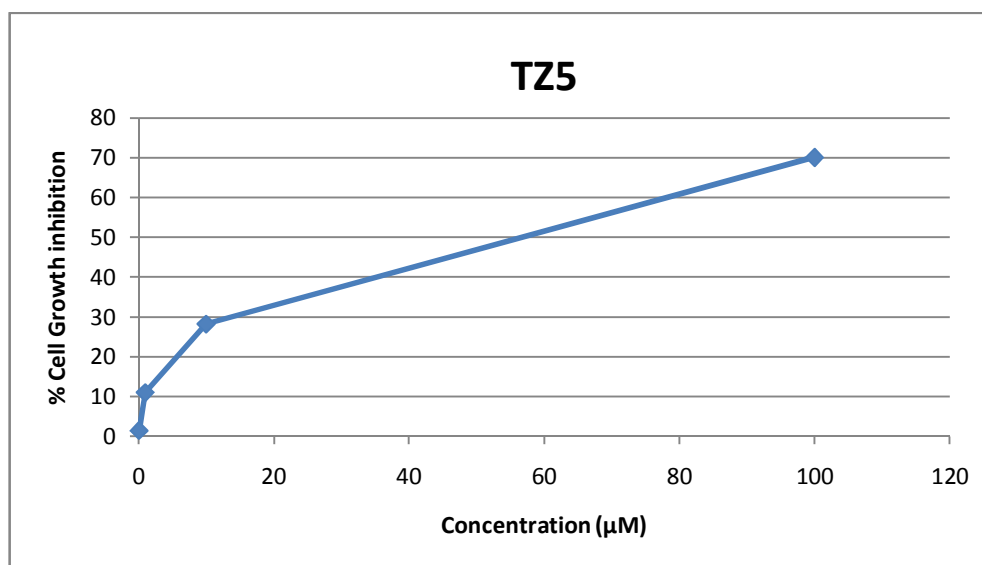


Table No. 9. TZ6

Concentration ( $\mu\text{M}$ )	% Growth inhibition	IC <sub>50</sub>	R <sup>2</sup>
0.1 $\mu\text{M}$	1.2342	48.60	0.9916
1 $\mu\text{M}$	12.3079		
10 $\mu\text{M}$	32.9861		
100 $\mu\text{M}$	73.8578		

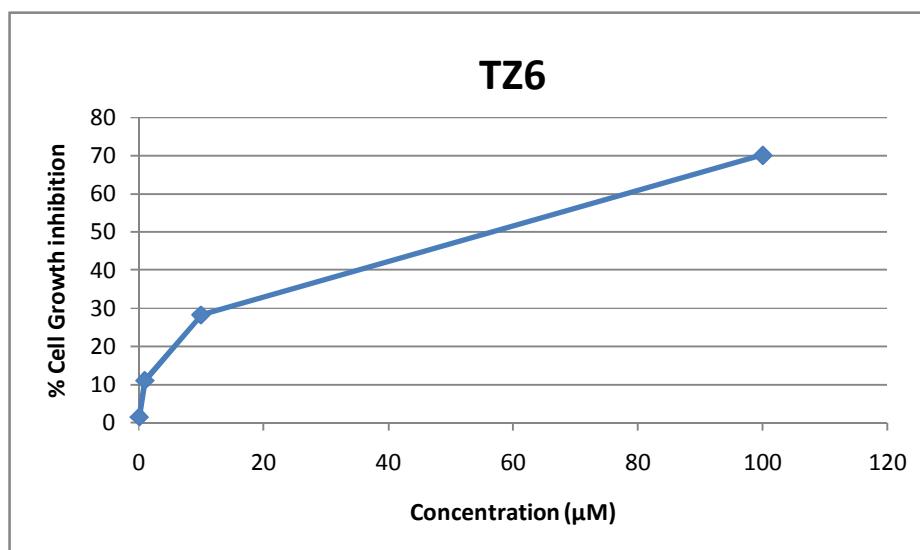


Table No. 10. TZ7

Concentration ( $\mu\text{M}$ )	% Growth inhibition	IC <sub>50</sub>	R <sup>2</sup>
0.1 $\mu\text{M}$	3.1895	75.26	0.9916
1 $\mu\text{M}$	14.2646		
10 $\mu\text{M}$	23.6772		
100 $\mu\text{M}$	55.0587		

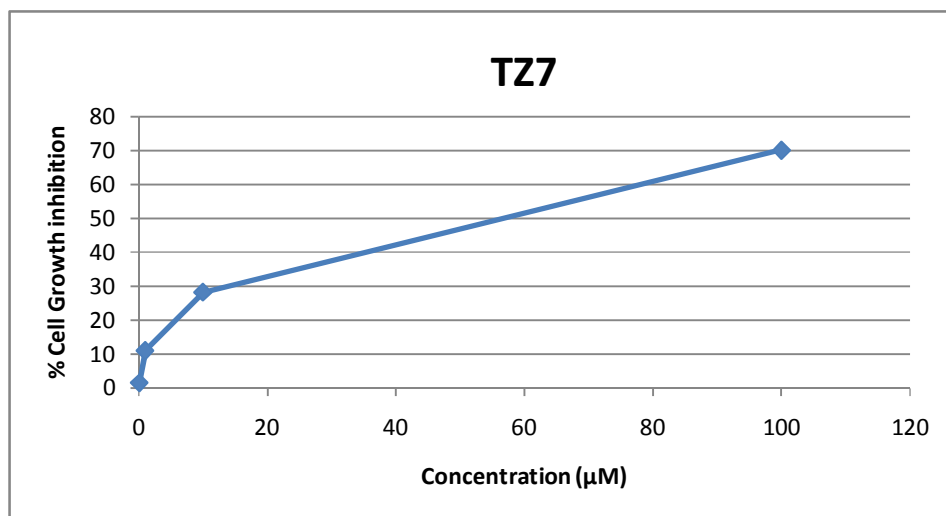


Table No. 11. TZ8

Concentration ( $\mu\text{M}$ )	% Growth inhibition	IC <sub>50</sub>	R <sup>2</sup>
0.1 $\mu\text{M}$	1.4315	92.36	0.9916
1 $\mu\text{M}$	19.6317		
10 $\mu\text{M}$	35.4864		
100 $\mu\text{M}$	60.8275		

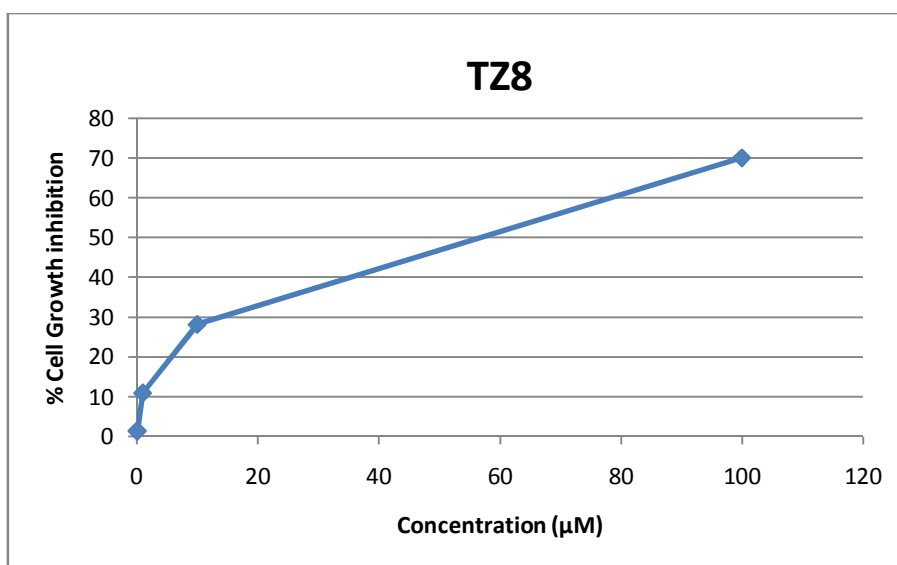


Table No. 12. TZ9

Concentration ( $\mu\text{M}$ )	% Growth inhibition	$\text{IC}_{50}$	$\text{R}^2$
0.1 $\mu\text{M}$	1.4846	70.48	0.9916
1 $\mu\text{M}$	11.3278		
10 $\mu\text{M}$	30.3398		
100 $\mu\text{M}$	60.0175		

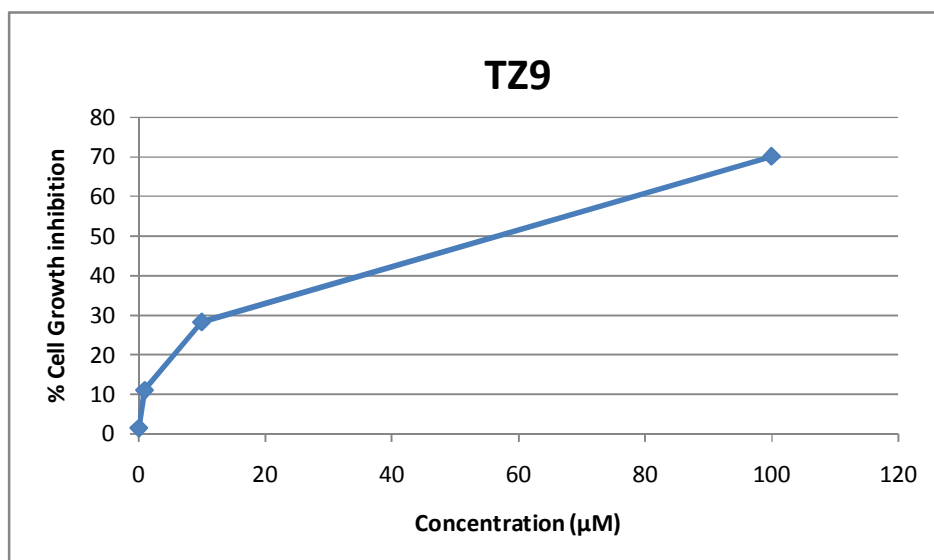
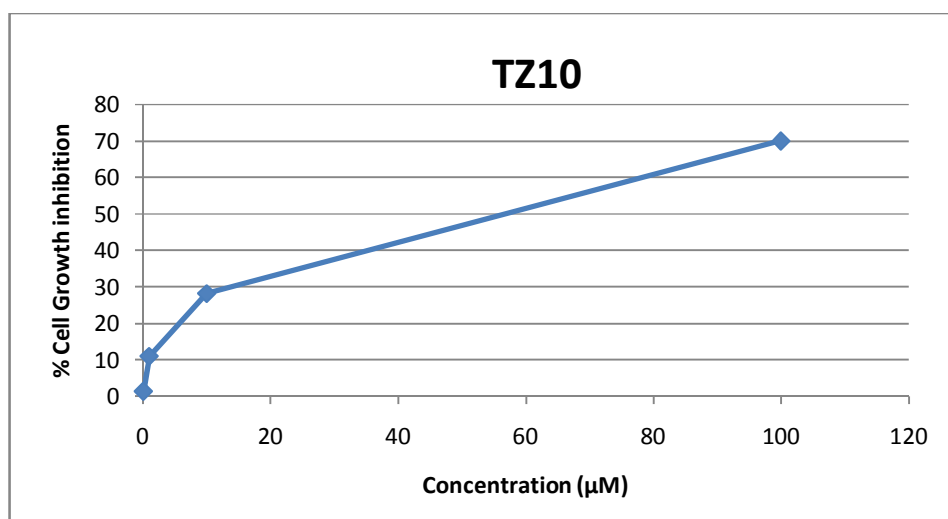


Table No. 12. TZ10

Concentration ( $\mu\text{M}$ )	% Growth inhibition	IC <sub>50</sub>	R <sup>2</sup>
0.1 $\mu\text{M}$	1.4579	49.26	0.9916
1 $\mu\text{M}$	11.0145		
10 $\mu\text{M}$	28.2358		
100 $\mu\text{M}$	70.1268		





## CHAPTER – 6

### SUMMARY AND CONCLUSION

In summary, a new series of substituted N-(5-aryl-1, 3, 4-thiadiazole-2-yl)-2-alkyl / aryl substituted acetamides were synthesized. These title compounds containing ten different substituents at C-2 and C-5 were screened for their anticancer agents. Most of the test compounds were found to exhibit significant anticancer activity against the human cervical cancer cell line (HeLa) in the highest concentration. Among the substituents at C-2, dimethylamino substituent and at C-5 4-methyl phenyl substituent showed maximum potency, while morpholino and pyrrolidine substituent showed equipotent activity but the piperidine substituent at C-2 exhibited least activity when compare to other substituents. The order of activity at C-2 is dimethylamino  $\geq$  morpholine  $\geq$  imidazole  $\geq$  pyrrolidine  $\geq$  piperidine group and at C-5 is 4-methyl  $\geq$  4-bromo group.

Among the test compounds, compound 2-(dimethylamino)-N-(5-p-tolyl-1,3,4-thiadiazol-2-yl)acetamide (**TZ1**) was found to be the most active agent which showed **74.85** percentage of cell inhibition against the human cervical cancer cell line (HeLa) in the highest concentration, which have dimethylamino group in the thiadiazole nucleus.

Hence this molecule can be selected as a lead molecule of the present study for further exploitation.

## **CHAPTER - 7**

### **FUTURE PLAN OF WORK**

It may conclude that further beneficial pharmacophore modifications in the design of novel 1, 3, 4-thiadiazole derivatives may be synthesized by designing novel ligands for therapeutic target by substituting different functional group and also examine with the help of NMR and X-ray which provide three dimensional frame works which can analyze structure activity data and can guide the design and synthesis of future potential therapeutic drugs towards other chronic disorders.

## CHAPTER- 8

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